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(71) Applicant *(for all designated States except US)*: **VANDERBILT UNIVERSITY** [US/US]; 1207 17th Avenue South, Suite 105, Nashville, TN 37212 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MARNETT, Lawrence, J.** [US/US]; 1884 Laurel Ridge Drive, Nashville, TN 37215 (US). **TIMOFEEVSKI, Sergei** [US/US]; 7790 Calle Mejor, Carlsbad, CA 92009 (US). **PRUDHOMME, Daniel** [US/US]; 4004 Westlawn Drive, Nashville, TN 37209 (US).

(74) Agent: **TAYLOR, Arles, A., Jr.**; Jenkins, Wilson & Taylor, P.A., Suite 1400 University Tower, 3100 Tower Boulevard, Durham, NC 27707 (US).

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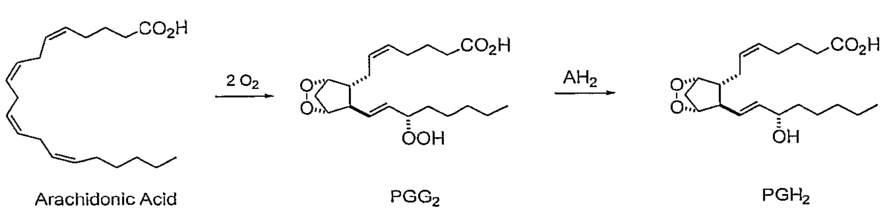
(54) Title: COX-2-TARGETED IMAGING AGENTS



(57) Abstract: The presently disclosed subject matter provides a method for synthesizing a radiological imaging agent by reacting a COX-2-selective ligand with a compound comprising a detectable group, wherein the COX-2- selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety. Also provided are compositions that are synthesized using the method, as well as methods of using the compositions of the presently disclosed subject matter.

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Description

COX-2-TARGETED IMAGING AGENTS

Cross Reference to Related Applications

This application is based on and claims priority to United States Provisional Application Serial Number 60/482,422, filed June 25, 2003, herein incorporated by reference in its entirety.

Grant Statement

This work was supported by grant CA85283 from the United States National Institutes of Health. Accordingly, the United States Government has certain rights in the presently disclosed subject matter.

Technical Field

The presently disclosed subject matter generally relates to imaging agents that comprise COX-2-selective ligands. More particularly, the presently disclosed subject matter relates to derivatives of non-steroidal anti-inflammatory drugs that exhibit binding to cyclooxygenase-2 (COX-2) and that comprise functional groups allowing them to be used as radiological imaging agents.

Table of Abbreviations

^{11}C	-	carbon-11
^{18}F	-	fluorine-18
ACN	-	acetonitrile
APC^{Min-}	-	a mouse strain that is highly susceptible to the formation of spontaneous intestinal adenomas
APHS	-	*o*-(acetoxyphenyl)hept-2-ynyl sulfide
At	-	astatine
BOC	-	*tert*-butoxycarbonyl
$(BOC)_2O$	-	Di-*tert*-butyl dicarbonate
Br	-	bromine
Cl	-	chlorine
COX-1	-	cyclooxygenase 1

COX-2	-	cyclooxygenase 2
CID	-	collision-induced dissociation
CT	-	computed tomography
DIPEA	-	diisopropylethylamine
DMAP	-	4-(dimethylamino)pyridine
DMF	-	dimethylformamide
DMSO	-	dimethyl sulfoxide
DOTA	-	tetraazacyclododecyltetraacetic acid
DTPA	-	diethylenetriamine pentaacetate
ED_{50}	-	effective dose 50
EDCI	-	1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide
ELISA	-	enzyme-linked immunosorbent assay
ESI	-	electrospray ionization
Et	-	ethyl
ETYA	-	5,8,11,14-eicosatetraynoic acid
F	-	fluorine
FAP	-	familial adenomatous polyposis
F-APHS	-	fluoroacetyl derivative of *o*-(acetoxyphenyl)hept-2-ynyl sulfide
FDA	-	U.S. Food and Drug Administration
$HCl_{(g)}$	-	HCl gas
HOBt	-	N-hydroxybenzotriazole
I	-	iodine
IC_{50}	-	concentration that inhibits by 50%
INDO	-	indomethacin
keV	-	kiloelectron volts
k_{inact}	-	rate constant for inactivation
K_i	-	inhibition constant
LAH	-	lithium aluminum hydride
LPS	-	lipopolysaccharide

MPM	-	mouse resident peritoneal macrophages
NIR	-	near infrared
NIH	-	National Institutes of Health
NMe_2	-	N,N-dimethyl
NMe_3	-	N,N,N-trimethyl
NSAIDs	-	non-steroidal anti-inflammatory drugs
PET	-	positron emission tomography
PG	-	prostaglandin
PGD_2	-	prostaglandin D_2
PGE_2	-	prostaglandin E_2
PGG_2	-	prostaglandin G_2
PGH_2	-	prostaglandin H_2
SPECT	-	single photon emission computed tomography
TEA	-	triethylamine
THF	-	tetrahydrofuran
TLC	-	thin layer chromatography
Ts-Cl	-	tosyl chloride
TXA_2	-	thromboxane A_2
TXB_2	-	thromboxane B_2

Background

A limitation of current diagnostic imaging methods is that it is often not possible to deliver the imaging agent specifically to the tissue or cell type that one wishes to image. In the case of target tissue imaging, what is needed is an agent that is specific for the target tissue, yet does not bind appreciably to surrounding non-target cells. Particularly desirable as imaging agents are compounds that can be used with non-invasive imaging techniques such as positron emission tomography (PET) and others.

In the area of diagnostic imaging of cancer, current methods for tumor-specific imaging are hindered by imaging agents that also accumulate in normal tissues. Additionally, a lack of targeting ligands that are capable of

binding to multiple tumor types necessitates the synthesis of a wide range of agents in order to image different tumor types. Ideally, a targeting molecule should display specific targeting in the absence of substantial binding to normal tissues, and a capacity for targeting to a variety of tumor types and stages. Finally, early diagnosis of neoplastic changes can result in more effective treatment of cancer. Thus, there exists a long-felt need in the art for methods to achieve delivery of imaging agents to tumors early in the course of tumorigenesis.

Cyclooxygenase (COX) activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2 (*see* DeWitt and Smith, 1988; Yokoyama and Tanabe, 1989; Hla and Neilson, 1992). COX-1 is a constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandin in the gastrointestinal tract and for the synthesis of thromboxane, which triggers aggregation of blood platelets (Allison *et al.*, 1992). COX-2, on the other hand, is inducible and short-lived. Its expression is stimulated in response to endotoxins, cytokines, and mitogens (Kujubu *et al.*, 1991; Lee *et al.*, 1992; O'Sullivan *et al.*, 1993).

Cyclooxygenase-2 (COX-2) catalyzes the committed step in the biosynthesis of prostaglandins, thromboxane, and prostacyclin (Smith *et al.*, 2000). COX-2 is not expressed in most normal tissues, but is present in inflammatory lesions and tumors (Fu *et al.*, 1990; Eberhart *et al.*, 1994). Studies by Eberhart *et al.* and Kargman *et al.* by first demonstrated that COX-2 mRNA and protein are expressed in tumor cells from colon cancer patients but not in surrounding normal tissue (Eberhart *et al.*, 1994; Kargman *et al.*, 1995). COX-2 expression appears to be an early event in colon tumorigenesis because it is detectable in colon polyps (Eberhart *et al.*, 1994). Approximately 55% of polyps demonstrate COX-2 expression compared to approximately 85% of colon adenocarcinomas. The concept that COX-2 is expressed in malignant tumors and their precursor lesions has been extended to a broader range of solid tumors including those of the esophagus (Kandil *et al.*, 2001), bladder (Ristimaki *et al.*, 2001), breast

(Ristimaki *et al.*, 2002), pancreas (Tucker *et al.*, 1999), lung (Soslow *et al.*, 2000), and melanoma (Denkert *et al.*, 2001).

The expression of COX-2 in tumors appears to have functional consequences. Prostaglandins have been demonstrated to stimulate cell proliferation (Marnett, 1992), inhibit apoptosis (Tsujii and DuBois, 1995), increase cell motility (Sheng *et al.*, 2001), and enhance angiogenesis in animal models (Daniel *et al.*, 1999; Masferrer *et al.*, 2000). COX-2 expression is dramatically elevated in rodent models of colon cancer and crossing COX-2 knockout mice into the APC^{Min-} background (a mouse strain that is highly susceptible to the formation of spontaneous intestinal adenomas) reduces the number of intestinal tumors by ~85% compared to APC^{Min-} controls (DuBois *et al.*, 1996; Oshima *et al.*, 1996). COX-2 expression is detected in breast cancers from the subset of patients exhibiting Her-2/neu overexpression. Overexpression of COX-2 specifically targeted to the breast of multiparous rodents induces breast cancer. These findings suggest that COX-2 contributes to tumor progression so that its expression in tumor tissue plays an important functional role. In fact, high COX-2 expression in tumors is associated with poor clinical outcome (Tucker *et al.*, 1999; Denkert *et al.*, 2001; Kandil *et al.*, 2001; Ristimaki *et al.*, 2002). Consequently, several clinical trials have been initiated to evaluate the potential of COX-2 inhibitors as chemopreventive agents and adjuvants to chemotherapy.

COX-2 is a molecular target for the anti-inflammatory, analgesic, and antipyretic effects of non-steroidal anti-inflammatory drugs (NSAIDs), particularly the recently developed COX-2-selective inhibitors, celecoxib (sold under the trade name CELEBREX® by Pfizer Inc. of New York, New York, United States of America) and rofecoxib (sold under the trade name VIOXX® by Merck and Co., Inc. of Whitehouse Station, New Jersey, United States of America). *See also* Vane and Botting, 1996. NSAIDs exhibit varying selectivity for COX-2 and COX-1 but, in general, few of them display high selectivity for COX-2 (Meade *et al.*, 1993). NSAIDs possess cancer

chemopreventive activity, while COX-selective drugs retard the growth of human tumor xenografts in nude mice and induce polyp regression in individuals with familial polyposis (Sheng *et al.*, 1997; Kawamori *et al.*, 1998; Steinbach *et al.*, 2000). These activities have been attributed to these drugs' ability to inhibit COX-2.

Summary

A method for synthesizing a radiological imaging agent is disclosed. In some embodiments, the method comprises reacting a COX-2-selective ligand with a compound comprising a detectable group, wherein the COX-2-selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety. In some embodiments, a carboxylic acid group of the NSAID has been derivatized to an ester or a secondary amine.

In some embodiments, the NSAID is selected from the group consisting of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments, the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, 6-methoxy-α-methyl-2-naphthylacetic acid, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolic acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zomepirac, and pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments, the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), ketorolac, and pharmaceutically acceptable salts thereof, and combinations thereof.

In some embodiments, the secondary amide derivative is selected from the group consisting of indomethacin-*N*-methyl amide, indomethacin-*N*-ethan-2-ol amide, indomethacin-*N*-octyl amide, indomethacin-*N*-nonyl amide, indomethacin-*N*-(2-methylbenzyl) amide, indomethacin-*N*-(4-methylbenzyl) amide, indomethacin-*N*-[(R)-α,4-dimethylbenzyl] amide, indomethacin-*N*-((S)-α,4-dimethylbenzyl) amide, indomethacin-*N*-(2-phenethyl) amide, indomethacin-*N*-(4-fluorophenyl) amide, indomethacin-*N*-(4-chlorophenyl) amide, indomethacin-*N*-(4-acetamidophenyl) amide, indomethacin-*N*-(4-methylmercapto)phenyl amide, indomethacin-*N*-(3-methylmercaptophenyl) amide, indomethacin-*N*-(4-methoxyphenyl) amide, indomethacin-*N*-(3-ethoxyphenyl) amide, indomethacin-*N*-(3,4,5-trimethoxyphenyl) amide, indomethacin-*N*-(3-pyridyl) amide, indomethacin-*N*-5-[(2-chloro)pyridyl] amide, indomethacin-*N*-5-[(1-ethyl)pyrazolo] amide, indomethacin-*N*-(3-chloropropyl) amide, indomethacin-*N*-methoxycarbonylmethyl amide, indomethacin-*N*-2-(2-L-methoxycarbonylethyl) amide, indomethacin-*N*-2-(2-D-methoxycarbonylethyl) amide, indomethacin-*N*-(4-methoxycarbonylbenzyl) amide, indomethacin-*N*-(4-methoxycarbonylmethylphenyl) amide, indomethacin-*N*-(2-pyrazinyl) amide, indomethacin-*N*-2-(4-methylthiazolyl) amide, indomethacin-*N*-(4-biphenyl) amide, and combinations thereof.

In some embodiments of the present method, the detectable group is selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof. In some embodiments, the halogen-containing moiety comprises a chloride atom, a fluorine atom, an iodine atom, a bromine atom, or a radioactive isotope thereof.

The presently disclosed subject matter also provides a method for imaging a target tissue in a subject. In some embodiments, the method comprises administering to the subject a radiological imaging agent under conditions sufficient for binding the radiological imaging agent to the target tissue, wherein the radiological imaging agent comprises a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or

a secondary amide moiety and further comprises a detectable group, and detecting the detectable group in the target tissue. In some embodiments of the method, a carboxyl group of the non-steroidal anti-inflammatory drug is derivatized to an ester or secondary amide.

In some embodiments, the target tissue is selected from the group consisting of an inflammatory lesion, a pre-neoplastic lesion, a tumor, a neoplastic cell, a pre-neoplastic cell, and a cancer cell. In some embodiments, the pre-neoplastic lesion is selected from the group consisting of a colon polyp and Barrett's esophagus. In some embodiments, the tumor is selected from the group consisting of a primary tumor, a metastasized tumor, and a carcinoma.

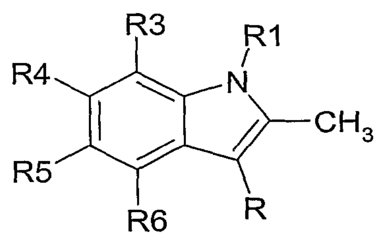
In some embodiments of the present method, the subject is a mammal. In some embodiments, the mammal is a human.

Various routes of administration of the imaging agent can be employed in the disclosed methods. In some embodiments, the administering is via a route selected from the group consisting of peroral, intravenous, intraperitoneal, inhalation, and intratumoral.

In some embodiments, the (NSAID) is selected from the group consisting of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments, the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, 6-methoxy-α-methyl-2-naphthylacetic acid, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolic acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zomepirac, and pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments,

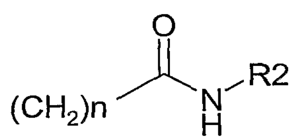
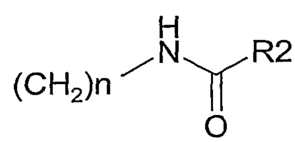
the NSAID is selected from the group consisting of aspirin, o-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), ketorolac, and pharmaceutically acceptable salts thereof, and combinations thereof.

The disclosed methods can employ radiological and/or optical imaging agents as disclosed herein. In some embodiments of the presently disclosed subject matter, the imaging agent comprises the following structure:

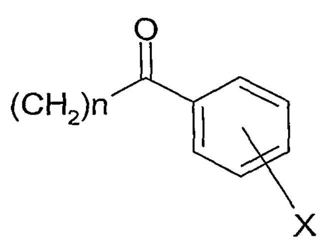
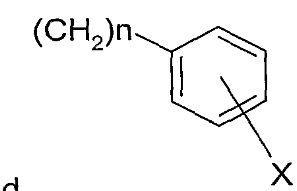


wherein

R is selected from the group consisting of

 and  ;

R1 is selected from the group consisting of a detectable group,

 and ,

wherein X is a halogen or a radioactive isotope thereof at one or more positions of the aromatic ring;

R2 comprises a detectable group or a halo substituted aryl;

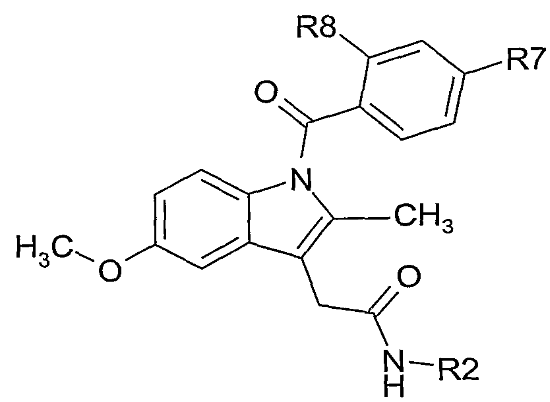
R3, R4, R5, and R6 are each independently selected from the group consisting of hydrogen; halo; C_1 to C_6 alkyl or branched

alkyl; C_1 to C_6 alkoxy or branched alkoxy; benzyloxy; SCH_3; $SOCH_3$; SO_2CH_3; SO_2NH_2; and $CONH_2$;

n is 0-5 inclusive;

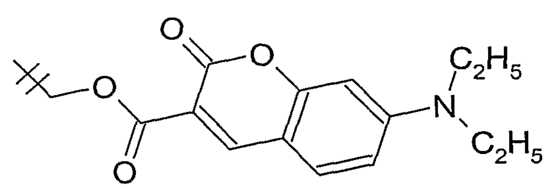
and wherein at least one of R1 and R2 comprises a detectable group.

In some embodiments, the imaging agent comprises the following structure:

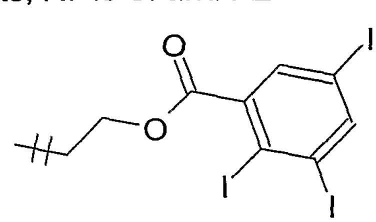


wherein R7 comprises a halogen and R8 is selected from the group consisting of hydrogen, a halogen, C_1-C_6 alkyl or branched alkyl, and C_1-C_6 aryl or branched aryl. In some embodiments, R3 is ^{18}F.

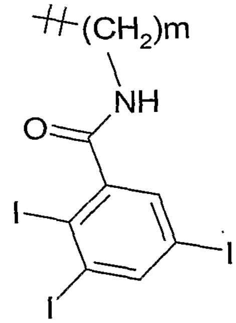
In some embodiments of the imaging agent, R7 is Cl and R2 has the following structure:



In some embodiments, R7 is Cl and R2 has the following structure:

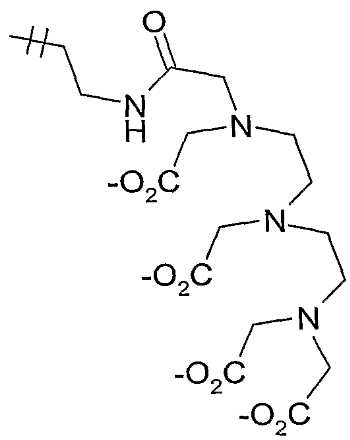


In some embodiments, R7 is Cl and R2 has the following structure:



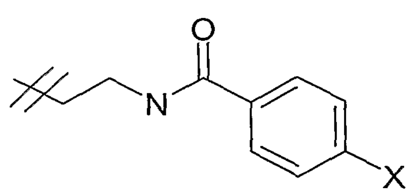
wherein m = an integer between 0 and 8, inclusive.

In some embodiments, R7 is Cl and R2 has the following structure:



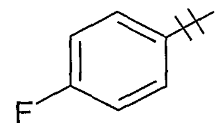
In some embodiments, R2 further comprises a coordinated metal ion. In some embodiments, the coordinated metal ion is selected from the group consisting of Gd^{3+}, Eu^{3+}, Fe^{3+}, Mn^{2+}, Yt^{3+}, Dy^{3+}, and Cr^{3+}. In some embodiments, the coordinated metal ion is Gd^{3+} or Eu^{3+}.

In some embodiments, R7 is Cl and R2 has the following structure:

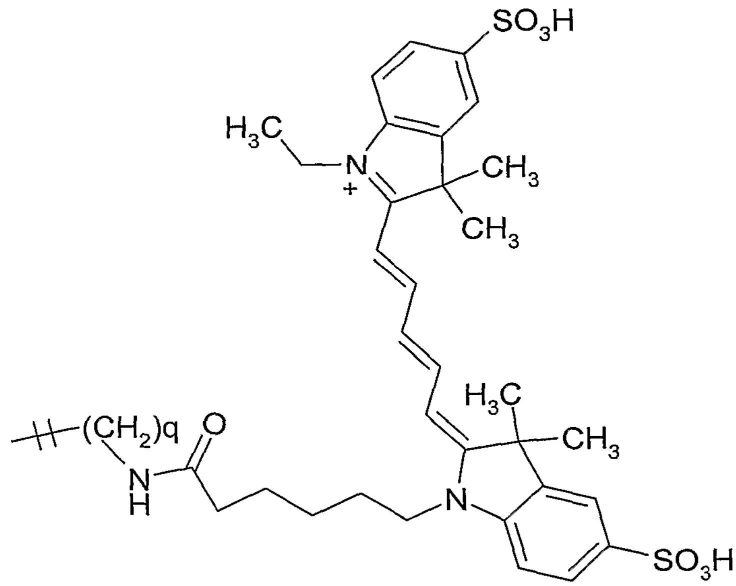


wherein X is a halogen or a radioactive isotope thereof. In some embodiments, X is ^{18}F.

In some embodiments, R7 is Cl and R2 has the following structure:

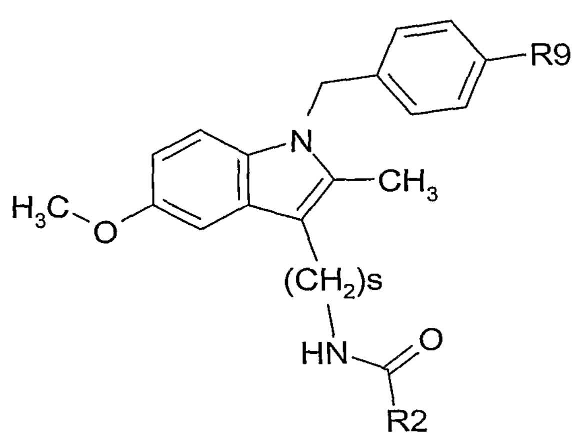


In some embodiments, R7 is Cl and R2 has the following structure:



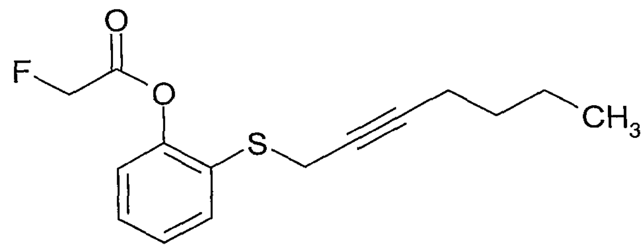
wherein q = an integer between 0 and 8, inclusive.

In some embodiments, the imaging agent comprises the following structure:



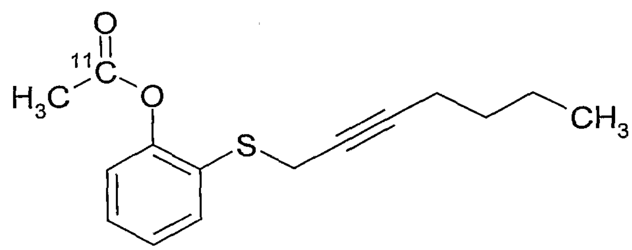
wherein R9 is a halogen, R2 is *p*-halobenzene, and s = 1-4. In some embodiments, R9 is Br, s = 2, and R2 is *p*-^{18}F-benzene.

In some embodiments, the imaging agent comprises the following structure:

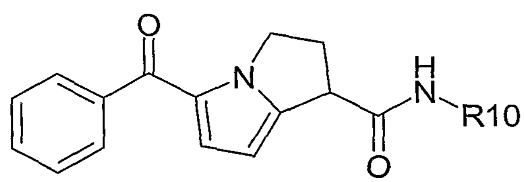


In some embodiments, the fluorine atom is ^{18}F.

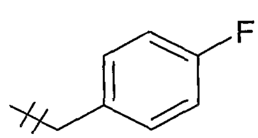
In some embodiments, the imaging agent comprises the following structure:



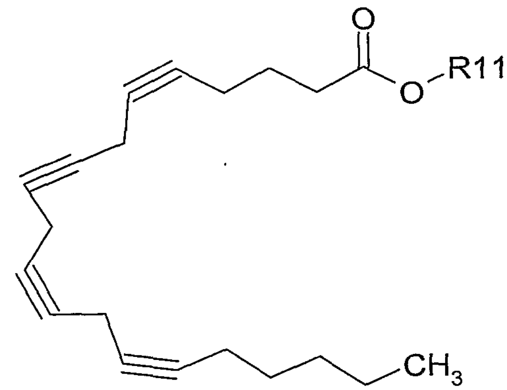
In some embodiments, the imaging agent comprises the following structure:



wherein R10 comprises a detectable group. In some embodiments of this aspect, R10 has the following structure:

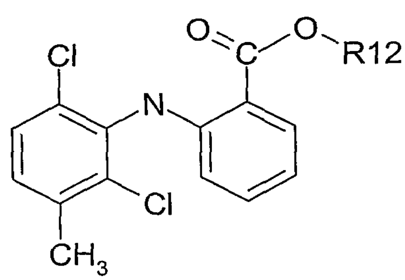


In some embodiments, the imaging agent comprises the following structure:



wherein R11 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

In some embodiments, the imaging agent comprises the following structure:

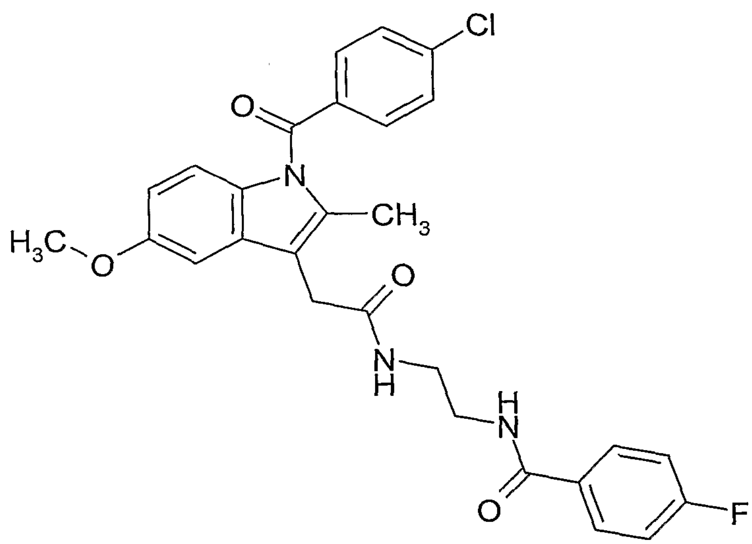


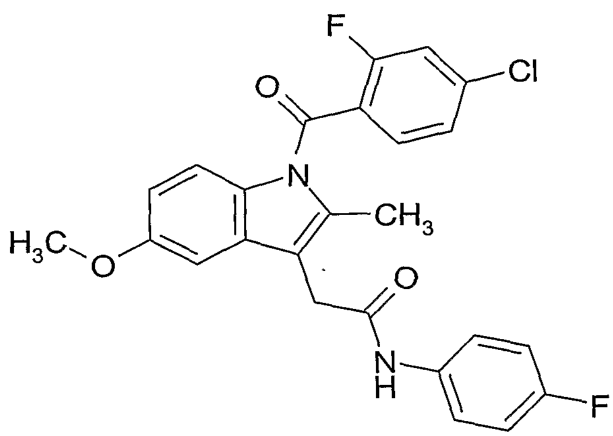
wherein R12 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

According to the present disclosure, the imaging agent comprises a detectable group. In some embodiments, the detectable group is selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof. The detectable group can be detected

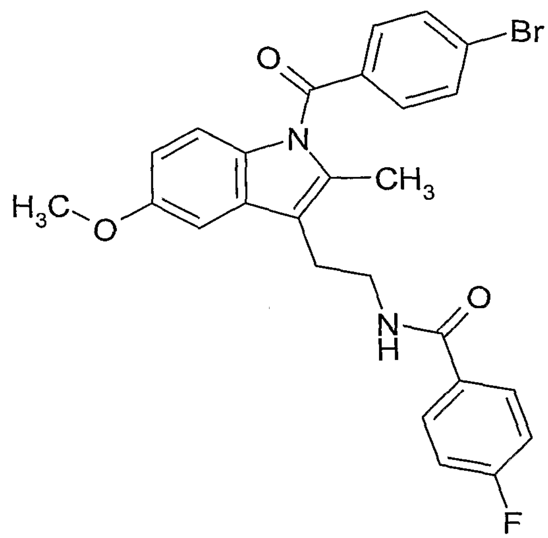
using various radiological and/or optical detection methodologies. In some embodiments, the detecting is by positron emission tomography, near infrared luminescence, or monochromatic X-ray.

The presently disclosed subject matter also provides an imaging agent comprising a detectable group and an indomethacin derivative, wherein the agent is selected from the group consisting of a compound having one of the following structures:





, and



In some embodiments, the detectable group comprises ^{18}F. In some embodiments, one or more fluorine atoms present in the structures listed above is ^{18}F.

Brief Description of the Drawings

Figure 1 depicts the general reaction catalyzed by cyclooxygenases by which arachidonic acid is converted to prostaglandin G_2 (PGG_2) and then to prostaglandin H_2 (PGH_2).

Figure 2 depicts the conversion of aspirin to *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS).

Figure 3 depicts the conversion of indomethacin to COX-2-selective ligands Compounds **1** and **2**.

Figure 4 depicts Compound **3**, a coumarin-derived ester of the ethanolamide of indomethacin.

Figure 5 depicts the structures of 5,8,11,14-eicosatetraynoic acid (ETYA), meclofenamic acid, ketorolac, and indomethacin, four NSAIDs to which the disclosed conversion process has been successfully applied.

Figure 6 depicts the structures of several indomethacin derivatives that bind to COX-2. None of the compounds shown inhibits COX-1 up to 66 μM.

Figure 7 depicts the synthesis of Compounds **4** and **5**, which are iodine-containing contrast agents. EDCI: 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide; DMAP: 4-(dimethylamino)pyridine.

Figure 8 depicts the synthesis of two iodine-containing contrast agents tethered via amide linkages of varying length. EDCI: 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide; HOBt: N-Hydroxybenzotriazole; DMF: dimethylformamide.

Figure 9 depicts an alternate synthesis scheme for the construction of iodine-containing contrast agents Compounds **8** and **10-12**. EDCI: 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide; DMAP: 4-(dimethylamino)pyridine; TEA: triethylamine; DMF: dimethylformamide; INDO: indomethacin.

Figure 10 depicts the synthesis of Compound **14**, a heavy metal chelating agent tethered to indomethacin.

Figure 11 depicts Compounds **16-18**, which are indomethacin derivatives.

Figure 12 depicts two alternative routes for the synthesis of ^{18}F-APHS. Et: ethyl group, CH_2CH_3.

Figure 13 depicts the synthesis of ^{11}C-APHS.

Figure 14 depicts the synthesis of ^{18}F-containing Compound **18**. Also shown are the fluorinated ketorolac and diarylpyrazole derivatives, Compounds **19** and **20**, respectively.

Figure 15 depicts the synthesis of indomethacin-based dyes for NIR luminescence imaging.

Figure 16 depicts a scheme for synthesizing indoyl amide derivatives of indomethacin, including a fluoro-standard, Compound **389**, and a PET precursor, Compound **390**. For each step, the components of each reaction are symbolized by an encircled lowercase letter. The components of each reaction are as follows: a: ammonium chloride, EDCI, HOBt, DIPEA, and DMF; b: LAH and THF; c: $(BOC)_2O$ and DMF; d: NaH, bromobenzylbromide, and DMF; e: $HCl_{(g)}$ and dichloromethane; f: 4-F-$C_6H_4CO_2H$, EDCI, HOBt,

DIPEA, and DMF; g: 4-NO_2-$C_6H_4CO_2H$, EDCI, HOBt, DIPEA, and DMF; h: KRYPTOFIX$_{2,2,2}$®, ^{18}F-KF, and ACN.

Figure 17 depicts a scheme for synthesizing various diamide derivatives of indomethacin. For each step, the components of each reaction are symbolized by an encircled lowercase letter. The components of each reaction are as follows: a: N-BOC-ethylenediamine, EDCI, HOBt, DIPEA, and DMF; b: $HCl_{(g)}$ and dichloromethane; c: EDCI, HOBt, DIPEA, and DMF (X = I, F, NO_2, OH, or NMe_2); d: $CF_3SO_3CH_3$ and dichloromethane; e: Ts-Cl and dichloromethane; f: KRYPTOFIX$_{2,2,2}$®, ^{18}F-KF, and ACN.

Figure 18 depicts a scheme for synthesizing amide derivatives of indomethacin. For each step, the components of each reaction are symbolized by an encircled lowercase letter. The components of each reaction are as follows: a: 10 N NaOH and DMF; b: 4-fluoroaniline, EDCI, HOBt, DMAP, and dichloromethane; c: NaH, 4-chloro-2-nitro-benzoyl chloride, and DMF; d: $SOCl_2$, pyridine, and DMF; e: NaH, 4-chloro-2-fluoro-benzoyl chloride, and DMF; f: KRYPTOFIX$_{2,2,2}$®, ^{18}F-KF, and ACN.

Figure 19 depicts a scheme for production of ^{18}F and the exchange chemistry that can be used to radiolabel NSAID (for example, indomethacin) derivatives to create COX-2-targeted imaging agents. For each step, the components of each reaction are symbolized by an encircled lowercase letter. The components of each reaction are as follows: a: $_{10}$KV bombardment; b: K_2CO_3; c: KRYPTOFIX$_{2,2,2}$®, DMSO, 85°C, with X = F, NO_2, I, OTs, or NMe_3^+.

Detailed Description

The present subject matter will be now be described more fully hereinafter with reference to the accompanying Examples, in which representative embodiments of the presently disclosed subject matter are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this

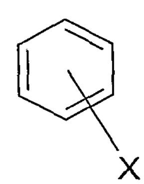
disclosure will be thorough and complete, and will fully convey the scope of the presently disclosed subject matter to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the presently disclosed subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers as well as racemic mixtures where such isomers and mixtures exist.

Throughout the specification, drawings, and claims, some chemical structures are depicted without including certain methyl groups and/or hydrogens. In the structures, solid lines represent bonds between two atoms, and unless otherwise indicated, between carbon atoms. Thus, bonds that have no atom specifically recited on one end and/or the other have a carbon atom at that and/or the other end. For example, a structure depicted as "– O –" represents C – O – C. Given that hydrogens are not explicitly placed in all structures, implicit hydrogens are understood to exist in the structures as necessary. Thus, a structure depicted as "– O" can represent H_3C – O, as appropriate given the valences of the particular atoms.

Additionally, throughout the specification, including the drawings and the claims, a bond that is depicted as such



is intended to represent an aromatic ring in which one or more of the hydrogens is replaced by another moiety, such as a halogen or a radioactive isotope thereof. As used herein, this schematic representation also represents aromatic rings in which more than one hydrogen has been

replaced. In those embodiments in which more than one hydrogen has been replaced, the schematic depiction is intended to represent any combination of different moieties (e.g. halogens and/or radioactive isotopes thereof) in any of the possible positions of the aromatic ring.

Following long-standing patent law convention, the terms "a" and "an" mean "one or more" when used in this application, including the claims.

I. General Considerations

Novel approaches have recently been developed that allow the facile conversion of non-selective NSAIDs into highly selective COX-2 ligands (Kalgutkar *et al.*, 1998a; Kalgutkar *et al.*, 2000a). This is accomplished by conversion of the carboxylic acid functional group, common to most NSAIDs, to a derivative. In one strategy, aspirin, an NSAID that covalently modifies COX-1 and COX-2 by acetylation, was converted to an acetylating agent, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), that is 100 times more selective for COX-2 than aspirin (Kalgutkar *et al.*, 1998a; *see also* Figure 2). Utilizing another strategy, it was discovered that several carboxylic acid-containing NSAIDs can be transformed into highly selective COX-2 inhibitors by converting them into neutral amide or ester derivatives (Kalgutkar *et al.*, 2000b). This strategy has proven effective in the case of the NSAIDs 5,8,11,14-eicosatetraynoic acid (ETYA), meclofenamic acid, ketorolac, and indomethacin (Figure 5). In the cases of ETYA, ketorolac, and meclofenamic acid, their amide derivatives exhibit selective COX-2 inhibitory activity. Several of the most potent inhibitors are haloalkyl or haloaryl amide derivatives, including the *p*-fluorobenzylamide of ketorolac (IC_{50}-COX-2 = 80 nM; IC_{50}-COX-1 > 65 μM) and the *p*-fluorophenylamide of indomethacin (IC_{50}-COX-2 = 52 nM; IC_{50}-COX-1 > 66 μM).

A major effort in the development of COX-2 inhibitors as derivatives of NSAIDs has focused on indomethacin as a parent compound. Indomethacin, which is approximately 15-fold more potent an inhibitor of COX-1 than COX-2, can be converted in a single step to amide or ester derivatives that exhibit COX-2 selectivities of greater than 1300-fold relative

to COX-1 (Figure 3; *see also* Kalgutkar *et al.*, 2000b). Both amides and esters of indomethacin are active, and a large number of alkyl and aromatic substituents exhibit potent and selective COX-2 inhibition. Figure 6 provides an example of some of the inhibitors that have been generated from the amidation of indomethacin, and illustrates the wide variety of structural moieties that are selective COX-2 inhibitors.

II. COX-2-Selective Ligands

In some embodiments, the presently disclosed subject matter relates to a method for synthesizing a radiological imaging agent comprising combining a COX-2-selective ligand with a functional group comprising a detectable moiety, wherein the COX-2-selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety. Thus, the method provides for the synthesis of a bifunctional molecule: one function being the ability to selectively bind COX-2, and the other to be detectable by radiological or optical imaging.

As used herein, the phrase "COX-2-selective ligand" refers to a molecule that exhibits preferential binding to a COX-2 polypeptide. As used herein, "selective binding" means a preferential binding of one molecule for another in a mixture of molecules. The binding of an inhibitor to a target molecule can be considered selective if the binding affinity is about 1×10^4 M^{-1} to about 1×10^6 M^{-1} or greater. In some embodiments, a COX-2-selective ligand is a COX-2-selective inhibitor, a "COX-2-selective inhibitor" being defined as a molecule that inhibits the activity of COX-2 in relative excess of its inhibition of COX-1. In some embodiments, COX-2-selective ligands bind covalently to COX-2 polypeptides. In other embodiments, COX-2-selective ligands bind non-covalently to COX-2 polypeptides

In some embodiments, a COX-2-selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID). As used herein, the term "derivative" refers to a structural variant of a compound in which one or more atoms have been changed to yield a new compound containing one or more functional groups that differ from the parent compound. This change can

occur by any suitable process, but typically occurs by reacting the NSAID with an intermediate, wherein a group is transferred from the intermediate to the NSAID to create a derivative.

NSAIDs that can be derivatized can intrinsically be COX-2 selective ligands. Alternatively, non-COX-2-selective NSAIDS can be converted into COX-2-selective ligands for use in the methods described herein. Methods for converting non-COX-2-selective NSAIDS into COX-2-selective ligands include the methods generally set forth in Kalgutkar *et al.*, 1998a; and/or Kalgutkar *et al.*, 1998b; and/or Kalgutkar *et al.*, 2000a; and/or Kalgutkar *et al.*, 2000b. These methods include, but are not limited to, methods for acetylating NSAIDs to make them COX-2-selective, and methods for converting NSAIDs into their respective neutral amide or ester derivatives to make them COX-2 selective. These methods are useful in making NSAID derivatives that covalently bind COX-2, as well as in making NSAID derivatives that non-covalently bind COX-2.

In some embodiments, the NSAID is selected from the group consisting of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments, the non-steroidal anti-inflammatory drug is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, 6-methoxy-α-methyl-2-naphthylacetic acid, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolic acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zomepirac, and pharmaceutically acceptable salts thereof, and combinations thereof. In some embodiments, the non-steroidal anti-inflammatory drug is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide

(APHS), indomethacin, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), ketorolac, and pharmaceutically acceptable salts thereof, and combinations thereof.

In some embodiments, a COX-2 ligand is a derivative of an NSAID comprising an ester moiety or a secondary amide moiety. In some embodiments, a carboxylic acid group of the NSAID as been derivatized to an ester or a secondary amide. In some embodiments, the secondary amide derivative is selected from the group consisting of indomethacin-*N*-methyl amide, indomethacin-*N*-ethan-2-ol amide, indomethacin-*N*-octyl amide, indomethacin-*N*-nonyl amide, indomethacin-*N*-(2-methylbenzyl) amide, indomethacin-*N*-(4-methylbenzyl) amide, indomethacin-*N*-[(R)-α,4-dimethylbenzyl] amide, indomethacin-*N*-((S)-α,4-dimethylbenzyl) amide, indomethacin-*N*-(2-phenethyl) amide, indomethacin-*N*-(4-fluorophenyl) amide, indomethacin-*N*-(4-chlorophenyl) amide, indomethacin-*N*-(4-acetamidophenyl) amide, indomethacin-*N*-(4-methylmercapto)phenyl amide, indomethacin-*N*-(3-methylmercaptophenyl) amide, indomethacin-*N*-(4-methoxyphenyl) amide, indomethacin-*N*-(3-ethoxyphenyl) amide, indomethacin-*N*-(3,4,5-trimethoxyphenyl) amide, indomethacin-*N*-(3-pyridyl) amide, indomethacin-*N*-5-[(2-chloro)pyridyl] amide, indomethacin-*N*-5-[(1-ethyl)pyrazolo] amide, indomethacin-*N*-(3-chloropropyl) amide, indomethacin-*N*-methoxycarbonylmethyl amide, indomethacin-*N*-2-(2-L-methoxycarbonylethyl) amide, indomethacin-*N*-2-(2-D-methoxycarbonylethyl) amide, indomethacin-*N*-(4-methoxycarbonylbenzyl) amide, indomethacin-*N*-(4-methoxycarbonylmethylphenyl) amide, indomethacin-*N*-(2-pyrazinyl) amide, indomethacin-*N*-2-(4-methylthiazolyl) amide, indomethacin-*N*-(4-biphenyl) amide, and combinations thereof.

Those skilled in the art will appreciate that an evaluation of the selectivity and efficacy of binding of the NSAID derivative to the COX-2 enzyme, *e.g.*, after the derivative is synthesized, can be desirable. Methods of screening selective COX-2 inhibitors for activity can be carried out *in vitro* and/or in intact cells, and are known in the art. *See e.g.*, Kalgutkar *et al.*,

1998a; Kalgutkar *et al.*, 1998b; Kalgutkar *et al.*, 2000a; Kalgutkar *et al.*, 2000b; Kalgutkar *et al.*, 2002. One example of an *in vitro* screening method takes advantage of the fact that both human and murine recombinant COX-2 can be expressed and isolated in pure form from an *Sf*-9 cell expression system. Briefly, typical assays involve the incubation of COX-1 (44 nM) or COX-2 (66 nM) in a 200 μL reaction mixture containing 100 mM Tris-HCl, pH 8.0, 500 μM phenol and 50 μM ^{14}C-arachidonic acid (55 mCi/mmol) for 30 seconds at 37°C. COX-1, which is not readily obtained in pure form from similar expression systems, can be purified from ovine seminal vesicles by standard procedures. Alternatively, membrane preparations from outdated human platelets can provide a source of human COX-1. The NSAID derivative(s) that is being screened for activity is added as a stock solution in dimethyl sulfoxide (DMSO) either concomitantly with the addition of arachidonic acid (to test for competitive inhibition) or for various periods of time prior to the addition of arachidonic acid (to test for time-dependent inhibition). The reaction is stopped by the addition of 200 μL of ethanol/methanol/1 M citrate, pH 4.0 (30:4:1). The extracted products are separated by thin layer chromatography (TLC), which allows quantitation of total product formation as well as assessment of product distribution. This assay is useful to define IC_{50} values for inhibition of either enzyme, and to determine time-dependency of inhibition. It also provides information concerning changes in products formed as a result of inhibition.

While the TLC assay described above provides considerable information, it is labor-intensive for screening large numbers of candidate NSAID derivatives. Accordingly, as an alternative, a simplified assay can be used. Incubation conditions can be essentially as described above, except all candidate derivatives are first screened at a concentration of 1 mM with a preincubation time of 30 minutes. The substrate need not be radiolabeled, and the reaction can be stopped by the addition of 2 μL of formic acid. Product formation can be quantitated by enzyme-linked immunosorbent assay (ELISA) using commercially available kits. Compounds found to

demonstrate potency and selectivity against COX-2 can optionally be further evaluated by the TLC assay. Other *in vitro* assay methods for screening NSAID derivatives for activity (*e.g.*, selectivity for the COX-2 enzyme) can also be used by the skilled artisan.

As will be appreciated by the skilled artisan, activity in purified enzyme preparations as described above does not guarantee that an NSAID derivative will be effective in intact cells. Thus, NSAID derivatives that are identified as potentially useful in the methods described herein can be further tested using, for example, the RAW264.7 murine macrophage cell line. These cells are readily available (for example, from the American Type Culture Collection, Manassas, Virginia, United States of America) and are easily cultured in large numbers. They normally express low levels of COX-1 and very low to undetectable levels of COX-2. Upon exposure to bacterial lipopolysaccharide (LPS), however, COX-2 levels increase dramatically over the ensuing 24 hour period, and the cells produce PGD_2 and PGE_2 from endogenous arachidonic acid stores (generally, ~1 nmol/10^7cells total PG formation). After LPS exposure, the addition of exogenous arachidonic acid results in the formation of additional PGD_2 and PGE_2 as a result of metabolism by the newly synthesized COX-2.

This system provides a number of approaches for testing the inhibitory potency of COX-2-selective ligands (*e.g.*, inhibitors). In general, following LPS activation, cells can be treated for 30 minutes with the desired concentrations of candidate derivative(s) in DMSO. ^{14}C-arachidonic acid can be added, and the cells can be incubated for 15 minutes at 37°C. Product formation can be assessed following extraction and TLC separation of the culture medium. Alternatively, the effects of candidate derivatives on PG synthesis from endogenous arachidonic acid can be assessed by incubating cells with desired concentrations of candidate derivatives 30 minutes prior to LPS exposure. Following a 24 hour incubation, medium can be collected and extracted, and the amount of PGD_2 and/or PGE_2 can be assayed by gas chromatography-mass spectrometry, liquid chromatography-

mass spectrometry, or ELISA. The latter method can prove to be particularly useful, since NSAID derivatives are often found to be more potent when assayed for activity using endogenous arachidonic acid as opposed to exogenously supplied substrate.

The RAW264.7 assay is but one example of a cell-based assay for screening the activity of NSAID derivatives; the skilled artisan will appreciate that assays using alternative cell lines and methodologies can be used.

III. Radiological and Optical Imaging Agents

Described herein are radiological and/or optical imaging agents that comprise COX-2-selective ligands and a detectable group. In certain embodiments, the COX-2-selective ligands are NSAID derivatives comprising an ester moiety or a secondary amide moiety. As used herein, the term "radiological imaging agent" refers to a compound that can be used to enhance the visualization of a tissue or cell using standard radiological or optical imaging techniques.

Methods of synthesizing inventive imaging agents are also described. In some embodiments, the present imaging agents are synthesized by reacting a COX-2-selective ligand with a compound comprising a detectable group. In certain embodiments, the COX-2-selective ligands are NSAID derivatives as described above. In still other certain embodiments, the NSAID derivatives comprise an ester moiety or a secondary amide moiety.

"Detectable groups", as defined herein, are functional groups that can be detected by one or more spectroscopic techniques, as described herein. Representative spectroscopic techniques that can be used to detect radiological and/or optical imaging agents and detectable groups include, but are not limited to, those techniques that detect fluorescence; chemical and biological luminescence; visible, ultraviolet, X-ray, infrared, and microwave light wavelengths; radiation generated by radioisotopes (for example, ^{18}F), and others. Specific techniques include, but are not limited to, scintigraphic imaging techniques (for example, positron emission tomography (PET),

single photon emission computed tomography (SPECT), gamma camera imaging, and rectilinear scanning), near infrared luminescence (NIR), and monochromatic X-ray.

The skilled artisan will appreciate that the selection of a particular spectroscopic technique plays a role in determining the desired characteristics of the imaging agent and detectable groups, and the applicability of any particular embodiment described herein to the selected technique. Stated another way, the skilled artisan will understand that the choice of a detectable group in synthesizing an imaging agent can depend in whole or in part on the specific spectroscopic technique being employed.

Exemplary detectable groups include, but are not limited to, halogen-containing moieties, fluorescent moieties, metal ion-chelating moieties, dyes, radioisotope-containing moieties, and combinations thereof. In some embodiments, a halogen-containing moiety comprises a fluorine atom, an iodine atom, a bromine atom, or a radioactive isotope thereof.

For use in positron emission tomography, the detectable group comprises an appropriate positron-emitting source. The term "positron-emitting source" refers to an atom that emits a particle that can directly or indirectly be detected using a PET scanner. PET generally uses a short half-life, radioactively labeled substance introduced into the material to be scanned (for example, into a tumor present within a subject) for the purposes of the scan. This radioactive substance emits positrons, which, after annihilation with electrons, give rise to positron annihilation radiation, which can be detected using standard PET techniques. Representative positron-emitting sources include, but are not limited to, ^{11}C, ^{14}O, ^{15}O, ^{17}F, ^{18}F, ^{19}Ne, ^{52}Fe, ^{62}Zn, ^{64}Cu, and ^{68}Ga, although other positron-emitting sources could also be employed.

For use in monochromatic X-ray detection, the detectable group will desirably comprise one or more iodine-containing moieties. Examples of such moieties include substituted benzene rings, in which at least one hydrogen has been replaced with iodine. In some embodiments, the iodine-

containing moiety comprises a benzene ring with three hydrogens replaced by iodine.

For use in fluorescent detection, the detectable can be a fluorescent dye (*e.g.*, a "fluorophore"). Many of these fluorescent dyes are commercially available, and include, but are not limited to, carbocyanine and aminostyryl dyes, long chain dialkyl carbocyanines (*e.g.*, DiI, DiO, and DiD available from Molecular Probes Inc., Eugene, Oregon, United States of America), and dialkylaminostyryl dyes.

A fluorescent label can also comprise sulfonated cyanine dyes, including Cy5, Cy5.5, and Cy7 (available from Amersham Biosciences Corp., Piscataway, New Jersey, United States of America), IRD41 and IRD700 (available from Li-Cor, Inc., Lincoln, Nebraska, United States of America), NIR-1 (available from Dejindo, Kumamoto, Japan), and LaJolla Blue (available from Diatron, Miami, Florida, United States of America). *See also* Licha *et al.*, 2000; Weissleder *et al.*, 1999; and Vinogradov *et al.*, 1996.

In addition, a fluorescent label can comprise an organic chelate derived from lanthanide ions, for example fluorescent chelates of terbium and europium. *See* U.S. Patent No. 5,928,627. Such labels can be conjugated or covalently linked to an NSAID derivative as disclosed therein. The chelator utilizes a number of coordinating atoms at coordination sites, as these terms are understood in the art, to bind the metal ion. The replacement of a coordination atom with a functional moiety to allow covalent attachment of the fluorescent label to a linker or other moiety might render the metal ion complex more toxic by decreasing the half-life of dissociation for the metal ion complex. Thus, in some embodiments, a site other than a coordination site is used for covalent attachment. However, for some applications, for example analysis of tumor tissue and the like, the toxicity of the metal ion complexes might not be of paramount importance and thus covalent attachment via a coordination site is appropriate.

Similarly, some metal ion complexes are so stable that even the replacement of one or more additional coordination atoms with a blocking

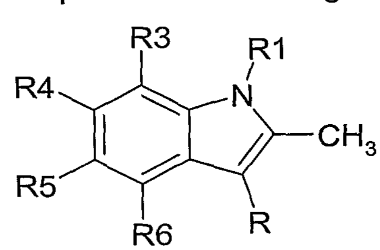
moiety does not significantly affect the half-life of dissociation. For example, both diethylenetriamine pentaacetate (DTPA) and tetraazacyclododecyltetraacetic acid (DOTA), described hereinbelow, are extremely stable when complexed with Gd^{3+}. Accordingly, one or several of the coordination atoms of the chelator can be replaced with one or more functional moieties for covalent attachment without a significant increase in toxicity.

There are a large number of known macrocyclic chelators or ligands that are used to chelate lanthanide and other metal ions. *See e.g.*, Alexander, 1995; Jackels, 1990, expressly incorporated herein by reference, which describes a large number of macrocyclic chelators and their synthesis. Similarly, there are a number of patents that describe suitable chelators for use in the invention, including U.S. Patent Nos. 5,155,215; 5,087,440; 5,219,553; 5,188,816; 4,885,363; 5,358,704; 5,262,532; and Meyer *et al.*, 1990, all of which are also expressly incorporated by reference. There are a variety of factors that influence the choice and stability of the chelate metal ion complex, including enthalpy and entropy effects (for example number, charge and basicity of coordinating groups, ligand field and conformational effects, etc.). In general, the chelator has a number of coordination atoms that are capable of binding the metal ion. The number of coordination atoms, and thus the structure of the chelator, depends on the metal ion. Thus, as will be understood by those in the art, any of the known metal ion chelators or lanthanide chelators can be easily modified using the teachings herein to add a functional moiety for covalent attachment to an optical dye or linker.

For *in vivo* detection of a fluorescent label, an image is created using emission and absorbance spectra that are appropriate for the particular label used. The image can be visualized, for example, by diffuse optical spectroscopy. Additional methods and imaging systems are described in U.S. Patent Nos. 5,865,754; 6,083,486; and 6,246,901, among other places.

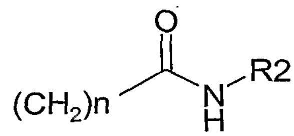
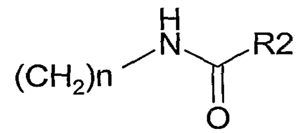
Near infrared (NIR) light that can penetrate tissue several centimeters, and fluorescent contrast agents responsive to NIR light can be used to provide a viable imaging system. For use in luminescent detection, the detectable group can be a chemical dye. Dyes that can be used include, but are not limited to, the class of polymethine dyes selected from the following group: cyanine, styryl, merocyanine, squaraine, and oxonol dyes. Representative dyes of the class of cyanine dyes having maximum absorption and fluorescence values between 700 and 1000 nm and extinction coefficients of about 140,000 l M^{-1} cm^{-1} and more, and carrying one or several unsubstituted, branched or non-branched, acyclic or cyclic or, optionally, aromatic carbon-hydrogen residues and/or containing oxygen, sulfur, nitrogen. For example, a dye can contain a cyanine, styryl, merocyanine, squaraine, or oxonol dye, or a mixture of said dyes. For example, cyanine dyes with intense absorption and emission in the near-infrared (NIR) region are particularly useful because biological tissues are optically transparent in this region (Wilson, 1991). For example, indocyanine green, which absorbs and emits in the NIR region, has been used for monitoring cardiac output, hepatic functions, and liver blood flow (He *et al.*, 1998; Caesar *et al.*, 1961), and its functionalized derivatives have been used to conjugate biomolecules for diagnostic purposes (Mujumdar *et al.*, 1993). *See also* U.S. Patent Nos. 5,453,505 and 6,403,625; WO 98/48846; WO 98/22146; WO 96/17628; WO 98/48838.

In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:

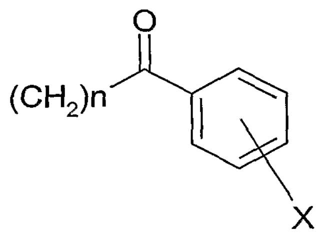
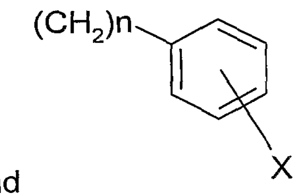


wherein

R is selected from the group consisting of

 and  ;

R1 is selected from the group consisting of a detectable group,

 and ,

wherein X is a halogen or a radioactive isotope thereof at one or more positions of the aromatic ring;

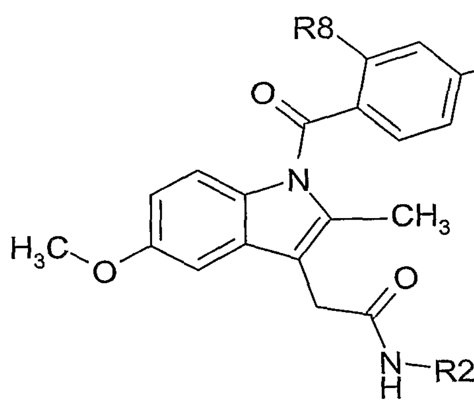
R2 comprises a detectable group or a halo substituted aryl;

R3, R4, R5, and R6 are each independently selected from the group consisting of hydrogen; halo; C_1 to C_6 alkyl or branched alkyl; C_1 to C_6 alkoxy or branched alkoxy; benzyloxy; SCH_3; $SOCH_3$; SO_2CH_3; SO_2NH_2; and $CONH_2$;

n is 0-5 inclusive;

and wherein at least one of R1 and R2 comprises a detectable group. Thus, n can be 0, 1, 2, 3, 4, or 5.

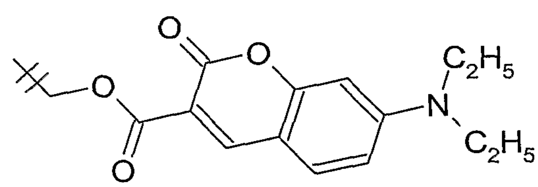
In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure



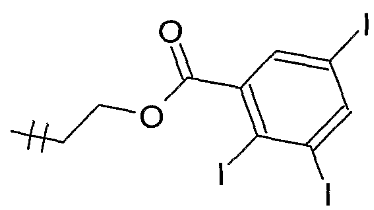
wherein R7 comprises a halogen and R8 is selected from the group consisting of hydrogen, a halogen, C_1-C_6 alkyl or branched alkyl, and C_1-C_6 aryl or branched aryl.

As used herein, the term "halogen" refers to one of the atoms of column VII of the Periodic Table of the Elements, and thus includes fluorine (F), chlorine (Cl), bromine (Br), iodine (I), and astatine (At). In some embodiments, a halogen is F, in some embodiments, a halogen is Cl, and in some embodiments a halogen is Br. As used herein, the term "halogen" refers to all isotopes of F, Cl, Br, I, and At including, but not limited to radioactive isotopes. In some embodiments, a halogen is ^{18}F.

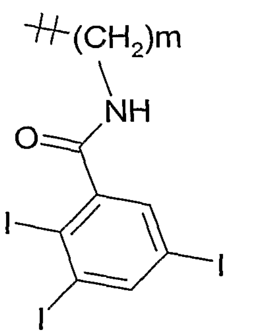
In some embodiments, R2 has the following structure:



In some embodiments, R2 has the following structure:

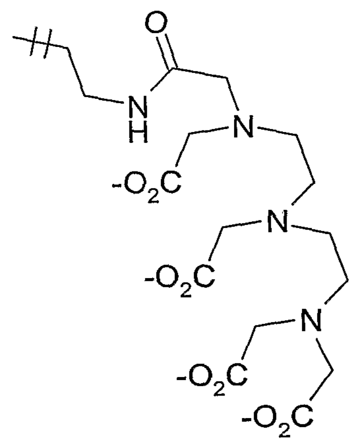


In some embodiments, R2 has the following structure:



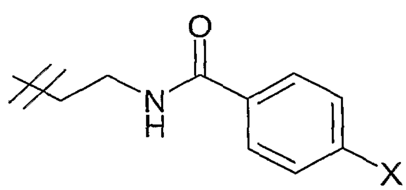
wherein m = an integer between 0 and 8, inclusive. Thus, m can be 0, 1, 2, 3, 4, 5, 6, 7, or 8.

In some embodiments, R2 has the following structure:



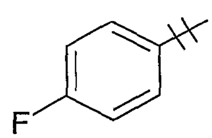
In some embodiments of this structure, the imaging agent further comprises a coordinated metal ion. In some embodiments, the coordinated metal ion is selected from the group consisting of Gd^{3+}, Fe^{3+}, Mn^{2+}, Yt^{3+}, Dy^{3+}, and Cr^{3+}. In some embodiments, the coordinated metal ion is Gd^{3}.

In some embodiments of the instant radiological imaging agent, R1 is Cl and R2 has the following structure:

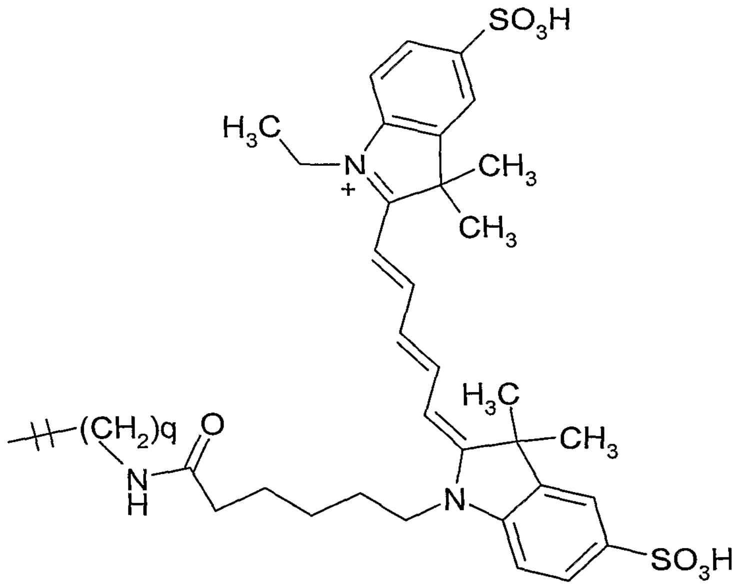


wherein X is a halogen or a radioactive isotope thereof. In some embodiments, X is ^{18}F.

In some embodiments of the present imaging agent, R2 has the following structure:

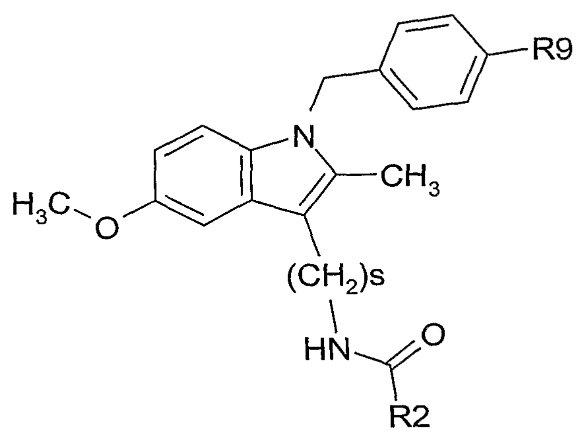


In some embodiments, R2 has the following structure:



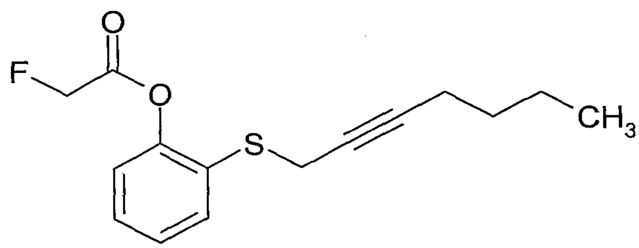
wherein q = an integer between 0 and 8, inclusive. Thus, q can be 0, 1, 2, 3, 4, 5, 6, 7, or 8.

In some embodiments, the radiological imaging agent comprises the following structure:



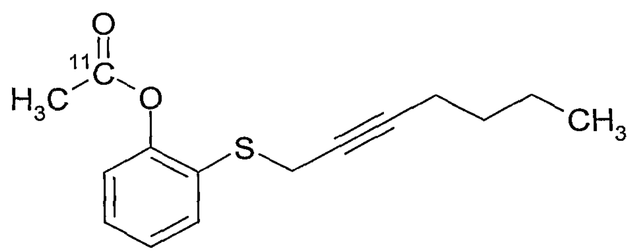
wherein R9 is a halogen, R2 is *p*-halobenzene, and s = 1-4. Thus, s can be 0,1,2,3, or 4. In some embodiments, R1 is Br, s = 2, and R2 is *p*-^{18}F-benzene.

In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:

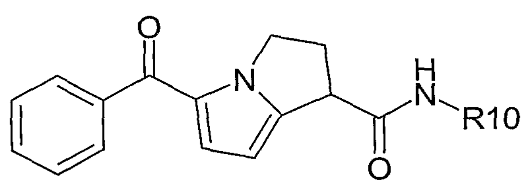


In some embodiments of the current radiological imaging agent, the fluorine atom is ^{18}F.

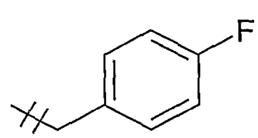
In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:



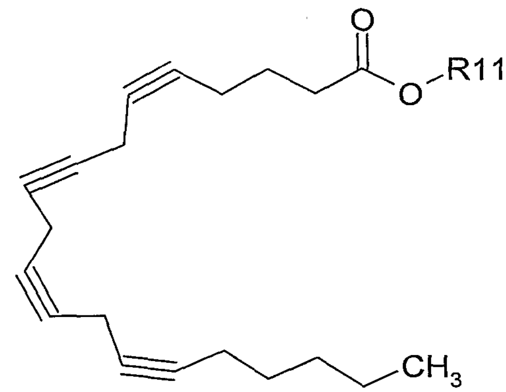
In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:



wherein R10 comprises a detectable group. In some embodiments, R10 has the following structure:

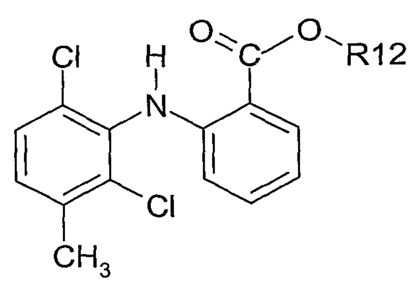


In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:



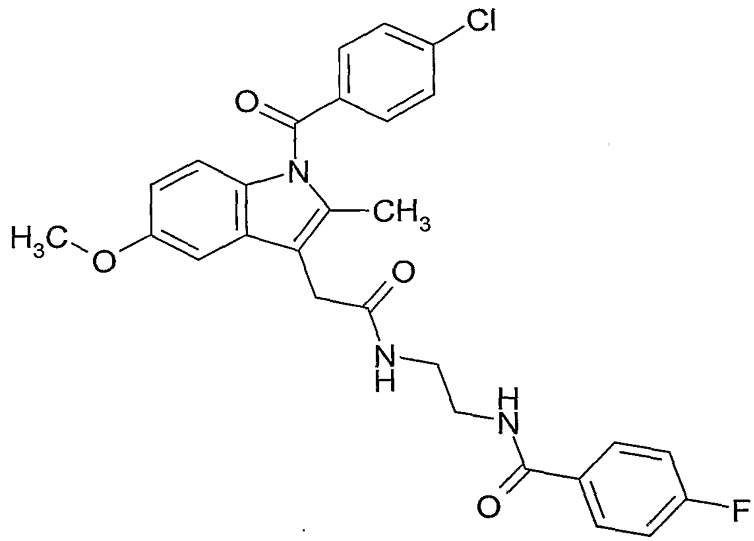
wherein R11 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

In some embodiments, a radiological imaging agent of the presently disclosed subject matter comprises the following structure:

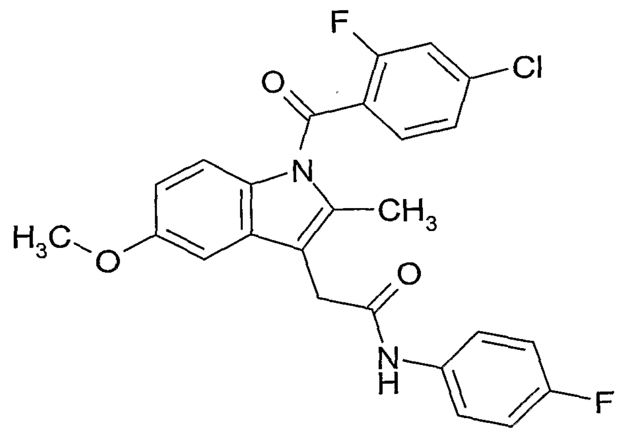


wherein R12 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

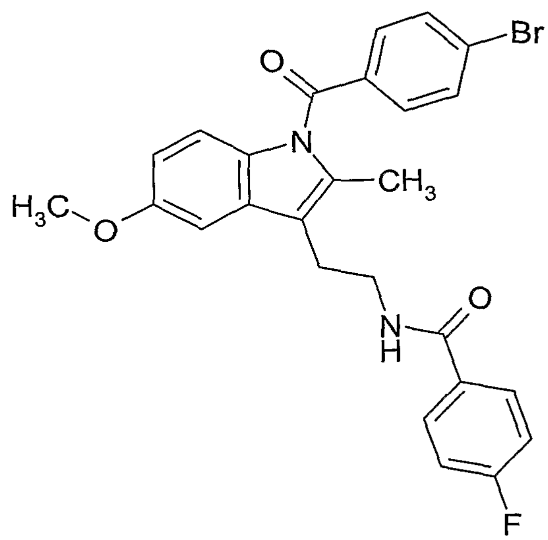
In some embodiments, the radiological imaging agent comprises a detectable group and an indomethacin derivative selected from the group consisting of Compounds **355**, **360**, and **389**, wherein Compounds **355**, **360**, and **389** have the following structures:



Compound **355**



Compound **360**



Compound **389**

In some embodiments of the instant radiological imaging agent, the detectable group is ^{18}F, and one or more fluorine atoms present in Compounds **355**, **360**, or **389** is ^{18}F.

Radiological imaging compounds described herein can optionally be evaluated by the skilled artisan for efficacy and suitability for a selected detection method. Such methods are known in the art and/or can be easily ascertained by the skilled artisan. For example, a synthesized radiological imaging compound can be evaluated as an imaging agent in intact cells. For such evaluations, mouse resident peritoneal macrophages (MPM) can be used. These cells normally possess relatively high quantities of COX-1, and low to undetectable quantities of COX-2 after isolation and overnight culture. However, following exposure to LPS, MPM show a rapid synthesis of COX-2 that begins within 1 hour and reaches a peak at 6 to 8 hours. Concomitantly, these cells produce large quantities of prostacyclin (identified as its decomposition product, 6-ketoPGF1a) and PGE_2. Thus, MPM respond to LPS more rapidly than do RAW264.7 cells, and produce larger quantities and different classes of PG products.

Quantitative western blot analysis of cell lysates have shown that after 6 hours of LPS treatment, MPM cells might contain as many as 10^5-10^6

molecules of COX-2 per cell, indicating a high concentration of the imaging target compound. Because COX-1 levels remain constant during this time, LPS-treated MPM contain both isoforms of the enzyme, whereas untreated MPM contain only COX-1. Thus, a comparison of the effects of imaging agents in LPS-treated versus untreated cells allows one to control for any effects due to binding to COX-1. Furthermore, mice bearing a targeted gene deletion of either the COX-1 or the COX-2 gene are available (S. K. Dey, Vanderbilt University, Nashville, Tennessee, United States of America; *see* Langenbach *et al.*, 1995; Morham *et al.*, 1995). MPM from these mice can serve as valuable controls to verify that effects of imaging agents are due specifically to COX-2.

MPM can be isolated from wild-type mice, or those bearing a targeted gene deletion by peritoneal lavage using well-established techniques. The cells are readily purified by adherence and cultured overnight. Following incubation for 6 hours in the presence or absence of LPS, cells can be treated for the desired period with inhibitors, then the appropriate imaging modality can be used to test the effectiveness of the test agent.

MPM-based screening assays can be tailored and optimized by the skilled artisan based on the kind of imaging agent being evaluated and the kind of detection technique being used. For example, radiological imaging agents comprising multiple iodine atoms for monochromatic X-ray can be tested. For the testing of these compounds, cells that have or have not been exposed to LPS can be treated with test compound, and then removed from the culture dishes and centrifuged, creating a cell button at the base of the centrifuge tube. Similar cultures of cells, which have not been exposed to the iodinated agent, can be treated identically. The tubes can then be suspended in a water phantom and 3-dimensionally imaged using the monochromatic X-ray beam tuned to the iodine k-edge (33.3 kiloelectron volts (keV)). Attenuation characteristics of the computed tomography (CT) images of the cell buttons can be established to determine whether or not the intracellular iodine has created a detectable signal to differentiate cells

exposed to inhibitor from those not exposed, and to differentiate LPS-treated from untreated cells.

Radiological imaging compounds synthesized for optical imaging techniques can similarly be evaluated. Briefly, cells are examined after treatment with candidate fluorescent or chelating agents. These cells can be examined in suspension (by spectroscopy) or after adhering to coverslips (microscopy). Quantitative measurements of fluorescence signals can be performed in the presence and absence of background (*i.e.* by adding untreated cells).

For PET imaging agents radiolabeled with ^{18}F, cells can be washed and scraped from culture dishes following incubation with inhibitors and the amount of radioactivity taken up can be determined by counting in an automated well scintillation γ-counter. Other screening methods for these agents can also be employed.

The *in vivo* efficacy of radiological imaging agents described herein can also be evaluated. For example, imaging agents can be evaluated for their ability to image COX-2-expressing tumors *in vivo*. Assays for this kind of evaluation are known in the art, and include, but are not limited to, the use of the HCA-7 human colon carcinoma xenograft model (*see e.g.,* Sheng *et al.*, 1997; Williams *et al.*, 2000b; Mann *et al.*, 2001); the murine Lewis lung carcinoma model (*see e.g,* Stolina *et al.*, 2000; Eli *et al.*, 2001); and murine colorectal carcinoma models that include, but are not limited to, the APC^{Min-} mouse model (*see* Su *et al.*, 1992; Moser *et al.*, 1995; Boolbol *et al.*, 1996; Williams *et al.*, 1996; Barnes and Lee, 1998; Jacoby *et al.*, 2000; Oshima *et al.*, 1996) and the azoxymethane-induced colon carcinoma model (Fukutake *et al.* 1998).

The term "independently selected" is used herein to indicate that the R groups, *e.g.*, R^1, R^2, R^3, etc. can be identical or different (*e.g.*, R^1, R^2 and R^3 can all be substituted alkyls, or R^1 and R^4 can be a substituted alkyl and R^3 can be an aryl, etc.). Moreover, "independently selected" means that in a multiplicity of R groups with the same name, each group can be identical to

or different from each other (*e.g.*, one R^1 can be an alkyl, while another R^1 group in the same compound can be aryl; one R^2 group can be H, while another R^2 group in the same compound can be alkyl, etc.).

A named R group will generally have the structure that is recognized in the art as corresponding to R groups having that name. For the purposes of illustration, representative R groups as enumerated above are defined herein. These definitions are intended to supplement and illustrate, not preclude, the definitions known to those of skill in the art.

As used herein, the term "alkyl" means C_{1-10} inclusive (i.e. carbon chains comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms; also, in some embodiments, C_{1-6} inclusive, i.e. carbon chains comprising 1, 2, 3, 4, 5, or 6 carbon atoms) linear, branched, or cyclic, saturated or unsaturated (*i.e.*, alkenyl and alkynyl) hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, hexyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, butadienyl, propynyl, butynyl, pentynyl, hexynyl, and allenyl groups.

The alkyl group can be optionally substituted with one or more alkyl group substituents which can be the same or different, where "alkyl group substituent" includes alkyl, halo, arylamino, acyl, hydroxy, aryloxy, alkoxyl, alkylthio, arylthio, aralkyloxy, aralkylthio, carboxy, alkoxycarbonyl, oxo and cycloalkyl. In this case, the alkyl can be referred to as a "substituted alkyl". Representative substituted alkyls include, for example, benzyl, trifluoromethyl, and the like. There can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, alkyl (also referred to herein as "alkylaminoalkyl"), or aryl. Thus, the term "alkyl" can also include esters and amides. "Branched" refers to an alkyl group in which an alkyl group, such as methyl, ethyl, or propyl, is attached to a linear alkyl chain.

The term "aryl" is used herein to refer to an aromatic substituent, which can be a single aromatic ring or multiple aromatic rings that are fused together, linked covalently, or linked to a common group such as a

methylene or ethylene moiety. The common linking group can also be a carbonyl as in benzophenone or oxygen as in diphenylether or nitrogen in diphenylamine. The aromatic ring(s) can include phenyl, naphthyl, biphenyl, diphenylether, diphenylamine, and benzophenone among others. In particular embodiments, the term “aryl” means a cyclic aromatic comprising about 5 to about 10 carbon atoms, including 5 and 6-membered hydrocarbon and heterocyclic aromatic rings.

An aryl group can be optionally substituted with one or more aryl group substituents which can be the same or different, where “aryl group substituent” includes alkyl, aryl, aralkyl, hydroxy, alkoxyl, aryloxy, aralkoxyl, carboxy, acyl, halo, nitro, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, acyloxyl, acylamino, aroylamino, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, arylthio, alkylthio, alkylene and –NR'R", where R' and R" can be each independently hydrogen, alkyl, aryl and aralkyl. In this case, the aryl can be referred to as a “substituted aryl”. Also, the term “aryl” can also included esters and amides related to the underlying aryl group.

Specific examples of aryl groups include but are not limited to cyclopentadienyl, phenyl, furan, thiophene, pyrrole, pyran, pyridine, imidazole, isothiazole, isoxazole, pyrazole, pyrazine, pyrimidine, and the like.

The term "alkoxy" is used herein to refer to the --OZ^1 radical, where Z^1 is selected from the group consisting of alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, heterocycloalkyl, substituted heterocycloalkyl, silyl groups and combinations thereof as described herein. Suitable alkoxy radicals include, for example, methoxy, ethoxy, benzyloxy, t-butoxy, etc. A related term is "aryloxy" where Z^1 is selected from the group consisting of aryl, substituted aryl, heteroaryl, substituted heteroaryl, and combinations thereof. Examples of suitable aryloxy radicals include phenoxy, substituted phenoxy, 2-pyridinoxy, 8-quinalinoxy, and the like.

The term "amino" is used herein to refer to the group --NZ^1Z^2, where each of Z^1 and Z^2 is independently selected from the group consisting of hydrogen; alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl,

heterocycloalkyl, substituted heterocycloalkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, alkoxy, aryloxy, silyl and combinations thereof. Additionally, the amino group can be represented as $N^+ Z^1 Z^2 Z^3$, with the previous definitions applying and Z^3 being either H or alkyl.

As used herein, the term "acyl" refers to an organic acid group wherein the -OH of the carboxyl group has been replaced with another substituent (i.e., as represented by RCO—, wherein R is an alkyl or an aryl group as defined herein). As such, the term "acyl" specifically includes arylacyl groups, such as an acetylfuran and a phenacyl group. Specific examples of acyl groups include acetyl and benzoyl.

"Aroyl" means an aryl-CO-- group wherein aryl is as previously described. Exemplary aroyl groups include benzoyl and 1- and 2-naphthoyl.

"Cyclic" and "cycloalkyl" refer to a non-aromatic mono- or multicyclic ring system of about 3 to about 10 carbon atoms, e.g., 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. The cycloalkyl group can be optionally partially unsaturated. The cycloalkyl group also can be optionally substituted with an alkyl group substituent as defined herein, oxo, and/or alkylene. There can be optionally inserted along the cyclic alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl, or aryl, thus providing a heterocyclic group. Representative monocyclic cycloalkyl rings include cyclopentyl, cyclohexyl, and cycloheptyl. Multicyclic cycloalkyl rings include adamantyl, octahydronaphthyl, decalin, camphor, camphane, and noradamantyl.

"Aralkyl" refers to an aryl–alkyl– group wherein aryl and alkyl are as previously described. Exemplary aralkyl groups include benzyl, phenylethyl, and naphthylmethyl.

"Aralkyloxyl" refers to an aralkyl–O– group wherein the aralkyl group is as previously described. An exemplary aralkyloxyl group is benzyloxyl.

"Dialkylamino" refers to an –NRR' group wherein each of R and R' is independently an alkyl group as previously described. Exemplary

alkylamino groups include ethylmethylamino, dimethylamino, and diethylamino.

"Alkoxycarbonyl" refers to an alkyl–O–CO– group. Exemplary alkoxycarbonyl groups include methoxycarbonyl, ethoxycarbonyl, butyloxycarbonyl, and t-butyloxycarbonyl.

"Aryloxycarbonyl" refers to an aryl–O–CO– group. Exemplary aryloxycarbonyl groups include phenoxy- and naphthoxy-carbonyl.

"Aralkoxycarbonyl" refers to an aralkyl–O–CO– group. An exemplary aralkoxycarbonyl group is benzyloxycarbonyl.

"Carbamoyl" refers to an H_2N–CO– group.

"Alkylcarbamoyl" refers to a R'RN–CO– group wherein one of R and R' is hydrogen and the other of R and R' is alkyl as previously described.

"Dialkylcarbamoyl" refers to a R'RN–CO– group wherein each of R and R' is independently alkyl as previously described.

"Acyloxyl" refers to an acyl–O– group wherein acyl is as previously described.

"Acylamino" refers to an acyl–NH– group wherein acyl is as previously described.

"Aroylamino" refers to an aroyl–NH– group wherein aroyl is as previously described.

The term "amino" refers to the $-NH_2$ group.

The term "carbonyl" refers to the –(C=O)– group.

The term "carboxyl" refers to the –COOH group.

The term "hydroxyl" refers to the –OH group.

The term "hydroxyalkyl" refers to an alkyl group substituted with an –OH group.

The term "mercapto" refers to the –SH group.

The term "oxo" refers to a compound described previously herein wherein a carbon atom is replaced by an oxygen atom.

The term "nitro" refers to the $-NO_2$ group.

The term "thio" refers to a compound described previously herein wherein a carbon or oxygen atom is replaced by a sulfur atom.

The term "sulfate" refers to the $-SO_4$ group.

IV. Indomethacin-based PET Contrast Agents

IV.A. General Considerations

The elevated expression of COX-2 in benign and malignant tumors and the apparent functional role that the enzyme plays in tumor growth suggests that COX-2 is an attractive target for the development of tumor-selective agents. The development of COX-2 selective indomethacin analogs has been accomplished by converting in one-step the non-selective COX-1 and COX-2 inhibitor indomethacin, into highly selective COX-2 inhibitors (*see* Kalgutkar *et al.*, 2000b). The enhanced selectivity results from the conversion of the carboxylic acid functionality into amides and esters. In some cases, derivatives exhibit COX-2 selectivity greater than 1000-fold over COX-1. Therefore, in some embodiments of the presently disclosed subject matter, the development of COX-2 selective imaging agents centered primarily on a 5-methoxy-2-methylindole core, the main constituent of indomethacin. Additional strategies for synthesizing indomethacin derivatives for use as starting materials for the production of indomethacin-based PET contrast agents are disclosed in U.S. Patent Nos. 6,207,700; 6,306,890; and 6,399,647.

In some embodiments, provided is the development of an indomethacin derivative PET agent. Positron emission tomography offers the highest spatial and temporal resolution of all nuclear medicine imaging modalities and allows quantitation of tracer concentrations in tissues. Of all the radioactive isotopes for PET, ^{18}F is the most practical to work with due to its relatively low positron emission energy (maximum 635 KeV) and shortest positron linear range in tissue (2.3 mm) resulting in the highest resolution in PET imaging. Furthermore, its half-life (109.8 min) is long compared to other

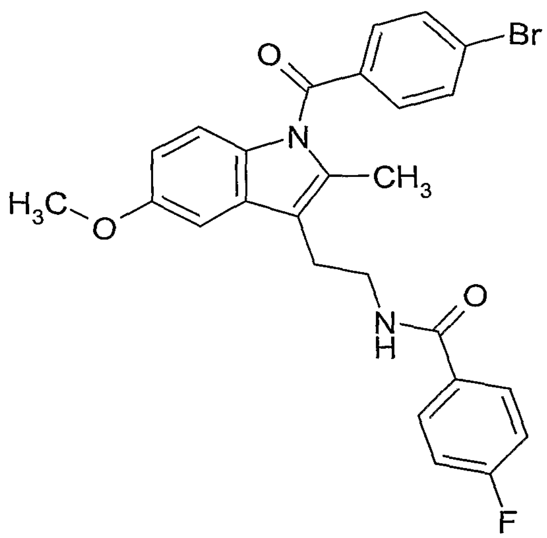
radioisotopes for relatively complex synthetic protocols and extended imaging sessions.

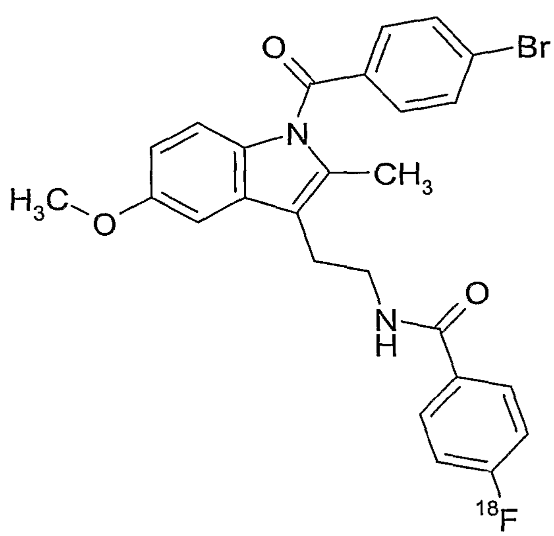
Despite the advantages of the modality, ^{18}F radionuclide synthesis is challenging due to ^{18}F's inherent half-life and radiation hazards. As such, all methods and manipulations of ^{18}F should be simple and ideally automatable. Optimally, the incorporation of the radioisotope should be at the end of the synthesis. For this reason, nucleophilic aromatic substitution is the method of choice for the incorporation of the ^{18}F anion into PET radioligand precursors. The exchange reaction is only possible, however, if activated (electron deficient) aromatics are used. Representative examples of suitable electron withdrawing groups on the aromatic moiety include the nitro, cyano, and carboxyl groups. Equally important is the presence of a suitable leaving group, with the trimethylammonium triflate salt being particularly useful.

Due to the short half-life of ^{18}F (2 hours), PET agents must be prepared such that the ^{18}F is incorporated at or near the end of the synthesis. Therefore, an ^{18}F precursor that is one step away from the final product is desirable. The precursors that have been designed incorporate known leaving groups that have proven to exchange with $^{18}F^-$ under the appropriate nucleophilic conditions of this reaction. The trimethylammonium triflate and tosylate are efficient precursors, with the nitro and halo groups also being useful.

IV.B. Indolyl Amide Series Indomethacin Derivatives

A generalized scheme for producing indomethacin derivatives in the indolyl amide series is shown in Figure 16. As shown in Figure 16 and described in Example 7, indomethacin can be converted through a series of steps to *N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-nitro-benzamide (Compound **389**). Compound **389** can then be labeled with ^{18}F using the strategy shown in Figure 19 to create a PET contrast agent that is specific for COX-2 (^{18}F-labeled Compound **389**).

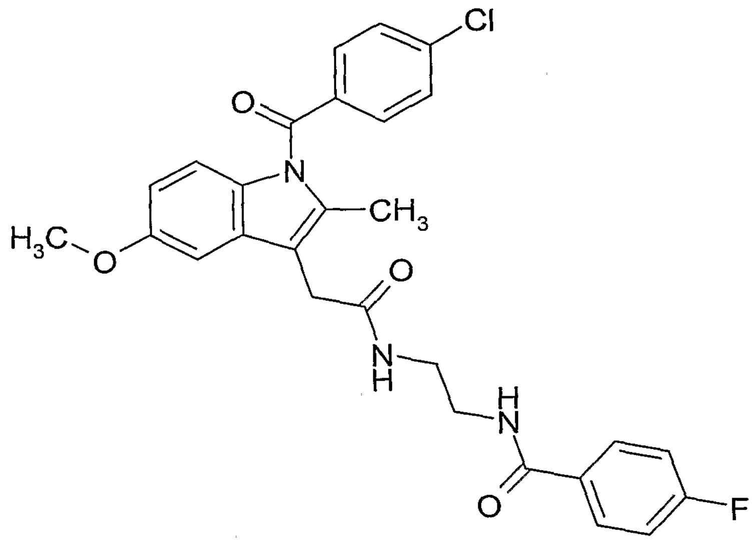




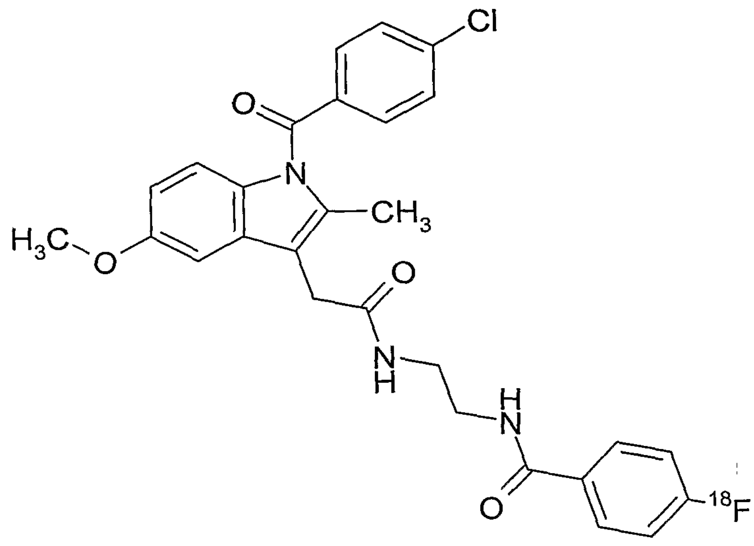
Compound **389** ^{18}F-labeled Compound **389**

IV.C. Diamide Series Indomethacin Derivatives

A generalized scheme for producing indomethacin derivatives in the diamide series is shown in Figure 17. As shown in Figure 17 and described in Example 8, indomethacin can be converted through a series of steps to *N*-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-4-fluoro-benzamide (Compound **355**). Compound **355** can then be labeled with ^{18}F using the strategy shown in Figure 19 to create a PET contrast agent that is specific for COX-2 (^{18}F-labeled Compound **355**).



Compound **355**

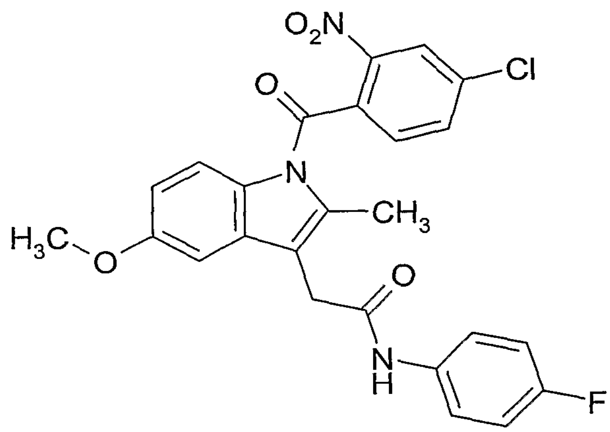


^{18}F-labeled Compound **355**

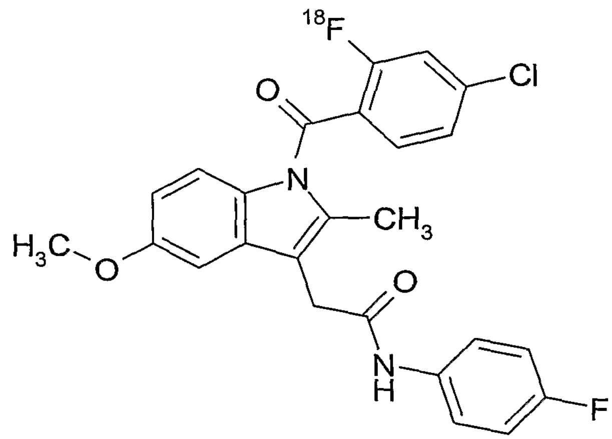
IV.D. Amide Series Indomethacin Derivatives

A generalized scheme for producing indomethacin derivatives in the amide series is shown in Figure 18. As shown in Figure 18 and described in Example 9, 5-methoxy-2-methyl-1*H*-indolacetic acid or Compound **360**, an indomethacin derivative synthesized by the co-inventors, can be converted through a series of steps to 2-[1-(4-Chloro-2-nitro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-(4-fluoro-phenyl)-acetamide (Compound **385**).

Compound **385** can then be labeled with ^{18}F using the strategy shown in Figure 19 to create a PET contrast agent that is specific for COX-2 (^{18}F-labeled Compound **360**).



Compound **385**



^{18}F-labeled Compound **360**

V. Methods of Use

The presently disclosed subject matter also includes methods for imaging a target tissue in a subject, the method comprising (a) administering to the subject a radiological imaging agent under conditions sufficient for binding of the radiological imaging agent to the target tissue, wherein the radiological imaging agent comprises a COX-2-selective derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a

secondary amide moiety and further comprises a detectable group; and (b) detecting the detectable group in the target tissue.

The term “target tissue” refers to any cell or group of cells present in a subject. This term includes single cells and populations of cells. The term includes, but is not limited to, cell populations comprising glands and organs such as skin, liver, heart, kidney, brain, pancreas, lung, stomach, and reproductive organs. It also includes, but is not limited to, mixed cell populations such as bone marrow. Further, it includes but is not limited to such abnormal cells as neoplastic or tumor cells, whether individually or as a part of solid or metastatic tumors. The term “target tissue” as used herein additionally refers to an intended site for accumulation of a ligand following administration to a subject. For example, the methods of the present invention employ a target tissue comprising a tumor. In some embodiments, the target tissue is selected from the group consisting of an inflammatory lesion, a tumor, a neoplastic cell, a pre-neoplastic cell, and a cancer cell. In some embodiments, the inflammatory lesion is selected from the group consisting of a colon polyp and Barrett’s esophagus.

As used herein, the term “cancer” encompasses cancers in all forms, including polyps, neoplastic cells, and pre-neoplastic cells.

As used herein, the term “neoplastic” is intended to refer to its ordinary meaning, namely aberrant growth characterized by abnormally rapid cellular proliferation. In general, the term “neoplastic” encompasses growth that can be either benign or malignant, or a combination of the two.

The term “tumor” as used herein encompasses both primary and metastasized solid tumors and carcinomas of any tissue in a subject, including but not limited to breast; colon; rectum; lung; oropharynx; hypopharynx; esophagus; stomach; pancreas; liver; gallbladder; bile ducts; small intestine; urinary tract including kidney, bladder and urothelium; female genital tract including cervix, uterus, ovaries (*e.g.,* choriocarcinoma and gestational trophoblastic disease); male genital tract including prostate, seminal vesicles, testes and germ cell tumors; endocrine glands including

thyroid, adrenal, and pituitary; skin (*e.g.*, hemangiomas and melanomas), bone or soft tissues; blood vessels (*e.g.*, Kaposi's sarcoma); brain, nerves, eyes, and meninges (*e.g.*, astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas and meningiomas). The term "tumor" also encompasses solid tumors arising from hematopoietic malignancies such as leukemias, including chloromas, plasmacytomas, plaques and tumors of mycosis fungoides and cutaneous T-cell lymphoma/leukemia, and lymphomas including both Hodgkin's and non-Hodgkin's lymphomas. The term "tumor" also encompasses radioresistant tumors, including radioresistant variants of any of the tumors listed above.

In some embodiments, the tumor is selected from the group consisting of a primary tumor, a metastasized tumor, and a carcinoma.

The methods and compositions of the presently claimed subject matter are useful for radiological imaging of a target tissue in any subject. Thus, the term "subject" as used herein includes any vertebrate species, for example, warm-blooded vertebrates such as mammals and birds. More particularly, the methods of the present invention are contemplated for the treatment of tumors in mammals such as humans, as well as those mammals of importance due to being endangered (such as Siberian tigers), of economic importance (animals raised on farms for consumption by humans) and/or social importance (animals kept as pets or in zoos) to humans, for instance, carnivores other than humans (such as cats and dogs), swine (pigs, hogs, and wild boars), ruminants and livestock (such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels), and horses. Also contemplated is the treatment of birds, including those kinds of birds that are endangered or kept in zoos, as well as fowl, and more particularly domesticated fowl or poultry, such as turkeys, chickens, ducks, geese, guinea fowl, and the like, as they are also of economic importance to humans. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human.

In some embodiments, the administering is peroral. In some embodiments, the administering is intravenous. In some embodiments, the administering is intraperitoneal. In some embodiments, the administration is intramuscular. In some embodiments, the administration is rectal. In some embodiments, the administration is by inhalation. In some embodiments, the administering is intratumoral. In some embodiments, a COX-2-selective ligand comprising a detectable group is administered intratumorally, and the tumor is visualized using PET.

Examples

The following Examples provide illustrative embodiments. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present inventors to work well in the practice of the embodiments. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

Example 1

Synthesis of Aspirin-Derived COX-2-Selective Ligands

Aspirin is a representative NSAID that has significant analgesic properties. It is the only NSAID that covalently modifies cyclooxygenases. Aspirin acetylates a serine residue (Ser530 of COX-1 and Ser516 of COX-2), which appears to block the active site of the enzyme for its substrates (Van der Ouderaa *et al.*, 1980; DeWitt *et al.*, 1990), thereby inactivating the enzyme. While aspirin acetylates both COX-1 and COX-2, it is about 10-100 times as potent against COX-1 as it is against COX-2 (Meade *et al.*, 1993; Vane and Botting, 1996).

Various derivatives of aspirin were investigated for their abilities to inhibit COX-1 and COX-2 in an effort to identify derivatives that displayed enhanced COX-2 inhibition relative to COX-1 inhibition. A series of acetoxybenzenes were derivatized in the ortho position with alkylsulfides. *o*-

(Acetoxyphenyl)methyl sulfide exhibited moderate inhibitory potency and selectivity for COX-2 (Kalgutkar *et al.*, 1998a). Variations in the acyl group, alkyl group, aryl substitution pattern, and heteroatom identity were also performed.

The compound that offered the best combination of potency and COX-2 selectivity was *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS). IC_{50} values for the inhibition of COX-2 and COX-1 by APHS are 0.8 μM and 17 μM, respectively. Like aspirin, APHS acetylates COX-2 at Ser516, and the time course for acetylation corresponds closely to the time course for irreversible inactivation of enzyme activity. Complete inactivation is achieved within about 30 min ($k_{inact}/K_i \sim 0.18\ min^{-1}\mu M^{-1}$). Consistent with the proposed mechanism of action, the S516A mutant of COX-2 is resistant to the inhibitory effects of APHS (Kalgutkar *et al.*, 1998a).

APHS is an effective inhibitor of COX-2 activity in the RAW 264.7 murine macrophage cell line activated by lipopolysaccharide (LPS) treatment. The IC_{50} for inhibition of PGD_2 synthesis in response to addition of exogenous arachidonic acid is 0.12 μM. Furthermore, APHS inhibits the growth in soft agar of HCA-7 colon cancer cells (IC_{50} = 2 μM), which express high levels of COX-2, and are dependent on COX-2 activity for maximal growth. In contrast, APHS has no effect on the growth of HCT-15 colon cancer cells, which do not express COX-2 (Kalgutkar *et al.*, 1998a).

Two *in vivo* models of inflammation have been used to assess the effectiveness of COX-2 selective inhibitors. The first is the rat carageenan footpad model. Maximal edema is obtained in this model 3 hours after carageenan injection. APHS inhibits edema formation with an ED_{50} of 6 mg/kg (p.o.). The ED_{50} for inhibition by aspirin is 125 mg/kg. APHS induces no gastric toxicity at doses of 100 mg/kg whereas 50% of the animals treated with 100 mg/kg aspirin develop gastric lesions.

The second model used to evaluate *in vivo* efficacy is the rat air pouch model. In this model, a subcutaneous air pouch is infused with carageenan to establish a local inflammatory response. PGE_2 produced in

the exudate is primarily the result of COX-2 activity, whereas thromboxane A_2 (TXA2) produced by blood platelets is the result of COX-1 activity. Thus, the selectivity of an inhibitor can be directly evaluated. In this model, APHS reduces PGE_2 levels in the pouch exudate by 95% at a dose of 5 mg/kg. This dose has no effect on serum thromboxane B_2 (TXB_2) levels. At a dose of 50 mg/kg, APHS reduces pouch PGE_2 and serum TXB_2 levels by 100% and 11%, respectively. These results contrast with those obtained with a 2 mg/kg dose of indomethacin, which reduces PGE_2 and TXB_2 levels by 100%, and 90%, respectively. Thus, APHS is a potent and selective COX-2 inhibitor *in vivo* (Kalgutkar *et al.*, 1998a). It is noteworthy that daily oral administration of APHS to Sprague-Dawley rats at a dose of 100 mg/kg induces no detectable toxicities at 14 days as judged by gross or histopathological evaluation.

Example 2

Fluoro Analogs of APHS

The ability of APHS to selectively acetylate COX-2 provides multiple opportunities for the design of a PET imaging agent. From a technical standpoint, the most easily accomplished is to synthesize an isotopically labeled haloalkyl derivative of APHS. This requires that such derivatives must be effective inhibitors of COX-2. To explore this possibility, a fluoroacetyl derivative of APHS (F-APHS) was synthesized and shown to be an effective inhibitor of COX-2 (IC_{50} = 4 μM). F-APHS inhibits the COX-2 activity in RAW 264.7 macrophages with an IC_{50} of 2.8 μM. However, it did not inhibit the COX-1 activity in uninduced macrophages at concentrations up to 32 μM.

Example 3

Radioactive Analogs of APHS

The fluorine atom of F-APHS can also be a radioactive isotope, such as ^{18}F. A direct synthesis route is a single-step exchange of $^{18}F^-$ for halogen, mesylate, or tosylate leaving groups. Previous reports indicate that $^{18}F^-$ exchanges with Br^- or I^- in bromo- or iodo-acetyl esters or with mesyl or

tosyl in mesyl- or tosyl-acetyl esters to form the corresponding ^{18}F-fluoroacetyl esters without hydrolysis (Figure 12; Block *et al.*, 1988). The iodo-derivative of APHS has been synthesized, and can be used for the exchange reaction.

Alternatively, an ^{18}F exchange with the tosyl-derivative of APHS can be used. The latter is available through tosylation of the glycolate ester of APHS. Tosylates are readily exchanged by F^-, so this method is a facile alternative in the event that exchange with iodo-APHS is undesirable (Block *et al.*, 1988).

One potential complication of the exchange reaction is hydrolysis of the acetyl-phenolate during ^{18}F exchange. Although this is considered unlikely, an alternative synthesis of ^{18}F-APHS has been designed in the event it occurs (Figure 12). Others have reported a two-step synthesis of ^{18}F-containing compounds in which $^{18}F^-$ exchange is performed on ethyl-bromoacetate then the ethyl-fluoroacetate is reacted with the nucleophilic center to be acylated (Tada *et al.*, 1990; Jalilian *et al.*, 2000). This two-step scheme has been used to make ^{18}F-fluoroacetyl amides and esters.

An alternate strategy for covalent imaging of COX-2 is to synthesize APHS labeled with ^{11}C in the acetyl group (Figure 13). Procedures have been described in which $^{11}CO_2$ is converted to ^{11}C-sodium acetate, which is rapidly purified by chromatography and solvent evaporation (Ishiwata *et al.*, 1995; van den Hoff *et al.*, 2001). The purified material is protonated and reacted with an excess of hydroxyphenylheptynylsulfide to directly produce ^{11}C-APHS. APHS is much less polar than either acetic acid or hydroxyphenylheptynylsulfide, so ^{11}C-APHS is purified by passage through a straight phase silica-based SEP-PAK™ matrix (Waters Corp., Milford, Massachusetts, United States of America). The ^{11}C-APHS elutes first from the column. The acetylation of hydroxyphenylheptynylsulfide is rapid as are the manipulations necessary for workup and purification.

Example 4

COX-2-Selective NSAID Derivatives as *In Vivo* Imaging Agents: Fluorescent Derivatives

Compound **3**, a coumarin-derived ester of the ethanolamide of indomethacin (*see* Figure 4) was synthesized according to the method of Timofeevski *et al.* (2002). This compound is very weakly fluorescent in buffer but yields a strong fluorescent signal on binding to COX-2. The signal is comprised of two components, a non-selective component exhibited on binding to both COX-1 and COX-2, and a selective component that is only observed with COX-2. The kinetics of the specific fluorescence increase corresponds exactly to those of the inhibition of COX-2 by the agent. Compound **3** binds to both apo- and holo-COX-2 but a COX-2-selective fluorescence increase is only observed with apo-protein. The heme prosthetic group of the holo-enzyme quenches the fluorescence.

While compound **3** would not be expected to be a highly successful imaging agent *in vivo* due to interference from hemoglobin in surrounding tissue, results obtained from these tests are useful in the construction of other fluorescent COX-2-selective optical imaging agents. These agents bind to holo-enzyme without loss in fluorescence, and exhibit minimal interference from hemoglobin or water allowing their use in cells and tissues. The selection of fluorophores having absorption and emission maxima at wavelengths in the near infrared (NIR) is ideal for this purpose, as these wavelengths fall between the absorption spectra of heme and water (Weissleder 2001).

Fluorinated indomethacin and ketorolac derivatives have been synthesized that are potent and highly selective COX-2 inhibitors. The *p*-fluorophenyl derivative of indomethacin amide (Compound **18**) and the *p*-fluorobenzyl derivative of ketorolac amide (Compound **19**) exhibit IC_{50} values of 52 nM and 80 nM, against purified COX-2, respectively. Compound **18** exhibits anti-inflammatory activity in the rat footpad edema assay following oral installation. Its bioavailability is 30% at a dose of 2 mg/kg and it has a 4

hr half-life in plasma following oral administration. Compound **19** has been shown that it is active in intact cells, inhibiting PGD_2 synthesis by LPS-activated RAW 264.7 cells with an IC_{50} of 200 nM.

Compounds **18** and **19** are synthesized with ^{18}F for PET imaging. In both cases, standard chemistry is employed in which *p*-trimethylammonium precursors are synthesized then exchanged with $^{18}F^{-}$ (Figure 14). Similar chemistry has been reported by McCarthy *et al.* for the synthesis of an ^{18}F-labeled COX-2 inhibitor of the diarylheterocycle class (Compound **20**) (McCarthy *et al.*, 2002). Compound **20** contains a *p*-methoxyphenyl group and a pyrazole group, which are similar to the *p*-methoxyindole group and the pyrrole group in 18 and 19. $^{18}F^{-}$ exchange has been successfully reported for compounds containing simple carboxylic acid esters, which are of comparable hydrolytic stability to the *p*-chlorobenzoyl group of 21. Hydrolysis of the *p*-chlorobenzoyl group of 21 is also carried out.

Fluorescent COX-2 inhibitors are also synthesized by coupling indomethacin to commercially available NIR fluorophores such as the succinimide esters Cy5, Cy5.5, and Cy7, supplied by Amersham Biosciences. The availability of the compounds with an activated carboxyl group provides an easy synthetic route to the desired inhibitors, by using indomethacin containing an amine linker. The structures of Cy5-indomethacin conjugates (Compounds **24** and **25**) are shown in Figure 15. The absorption and emission maxima of Cy5 are 650 nm and 668 nm, respectively. Cy5.5 and Cy7 have maxima at longer wavelengths. Molecular Probes also offers a series of NIR fluorophores available as succinimide esters. These compounds, Alexa 647, 660, 680, 700, and 750, have absorption and emission maxima that range from 650 nm to 780 nm, thus encompassing the entire NIR spectrum. They also offer higher extinction coefficients and greater stabilities than the Cy series of dyes.

Example 5

COX-2-Selective NSAID Derivatives as *In Vivo* Imaging Agents: Iodine-containing Agents

Several approaches have been used to synthesize iodine-containing X-ray contrast agents. The esterification of the ethanolamide of indomethacin has been accomplished by carbodiimide coupling of indomethacin ethanolamide (Compound **4**) and 2,3,5-triiodobenzoic acid (Figure 7). The product, Compound **5**, is a potent and highly selective COX-2 inhibitor (IC_{50} for COX-2 = 50 nM, IC_{50} for COX-1 > 50 μM). Higher concentrations are required for inhibition of COX-2 in the RAW264.7 macrophage cell line (IC_{50} = 3.5 μM), which might be related to the hydrophobicity of the compound (cLogP = 8.5). Amide derivatives (Compounds **8** and **9**) that correspond to the ester, Compound **5**, are generated. Compounds **6** and **7** are synthesized and their coupling to 2,3,5-triiodobenzoic acid is carried out

In addition to the straightforward coupling outlined in Figure 8, the alternate strategy outlined in Figure 9 can also be used to produce an iodine-containing NSAID. The scheme in Figure 9 has the advantage of generating the nucleophilic primary amine under conditions that do not expose the base-labile *p*-chlorobenzoyl group of the indomethacin moiety to strong base.

Example 6

COX-2-Specific NSAID Derivatives as *In Vivo* Imaging Agents: Chelating Agents

Radiological and/or optical imaging agents comprising heavy metal chelating derivatives of NSAIDs are synthesized. The diethyltriaminepentaacetic acid conjugate to Compound **6** as well as its Gd^{3+} derivative, Compound **15**, have been synthesized (*see* Figure 10). The use of an excess of the DTPA dianhydride, Compound **13**, generated the desired product cleanly and efficiently. Purification of the product was accomplished by reverse phase silica gel chromatography. Gd^{3+} was successfully added

to the chelator by dissolving the hexahydrate chloride salt in water, and successful incorporation was confirmed by mass spectrometry. The uncomplexed chelator, Compound **14**, displayed no inhibitory activity against COX-2 or COX-1 whereas the Gd^{3+} derivative, Compound **15**, exhibited weak COX-2 inhibition.

Materials and Methods for Examples 7-9

All reactions were performed under an atmosphere of ultra high purity argon. Commercially obtained chemicals were used as received. Reactions were monitored using thin layer chromatography (TLC) plates (Silica Gel 60 F_{254} precoated, 20 X 10 cm, 0.25 mm) from Analtech, Inc. (Newark, Delaware, United States of America). Purification was performed on column chromatography using silica followed by recrystalization from EtOAc/hexanes. ^{1}H and ^{13}C NMR data were recorded on a Bruker AC-300 NMR System (Bruker Bio-Spin Corp., Billerica, Massachusetts, United States of America) at 300 and 75 MHz, respectively, in $CDCl_3$ unless otherwise noted. Chemical shifts are reported in parts per million (ppm) downfield from TMS (δ = 0); coupling constants are given in hertz. Positive ion channel electrospray ionization (ESI) and collision-induced dissociation (CID) mass spectra were obtained on a Finnigan TSQ 7000 mass spectrometer (Thermo Electron Corp., Waltham, Massachusetts, United States of America).

Example 7

Synthesis of Indolyl Amides of Indomethacin

Indolyl amides of indomethacin were synthesized using the general scheme outlined in Figure 16.

2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetamide (Compound **301**). Indomethacin (3.5 g, 0.010 mol) and hydroxybenzotriazole (2 g, 0.015 mol) were dissolved in DMF (100 ml). To the mixture was added ammonia in dioxane, 0.5 M (50 ml, 0.025 mol). The mixture was cooled to 0°C and 1-cyclohexyl-3-(2-morpholino-ethyl)carbodiimide metho-p-toluenesulfonate (5 g, 0.012 mol) was added.

The reaction was stirred overnight and allowed to warm to room temperature. All solvents were removed via high vacuum and residue was taken up in ethylacetate (1200 ml) and brine (500 ml). The reaction was partitioned between two 1000 ml Erlenmeyer flasks for ease of handling. Mixtures were heated to completely dissolve all solids. The organic layer was washed with NaOH (1 N, 6 X 30 mL) to remove all traces of indomethacin. Yield, 95%; ^1H NMR (MeOH-d_4) δ 7.72-7.63 (m, 4H), 7.45 (s, 1H), 7.12 (s, 1H), 6.97-6.91 (m, 2H), 6.72-6.69 (m, 1H), 3.77 (s, 3H), 3.47 (s, 2H), 2.23 (s, 3H); ^{13}C NMR (MeOH-d_4) 171.9, 168.2, 155.9, 137.9, 135.5, 134.6, 131.5, 131.3, 130.7, 129.4, 114.9, 111.5, 102.3, 55.8, 31.3, 13.7; ESI-CID 379 (MNa^+), *m/z* 298, 89, 23.

<u>5-methoxy-2-methyl-3-indolacetamide (Compound **303**)</u>. Compound **301** (3.5 g, 9.8 mmol) was dissolved in dry DMF (100 mL) and stirred at room temperature. NaOH (10 N, 20 mL) was slowly added in small quantities over 1 hour while monitoring the reaction by TLC. The reaction was judged complete after 2 hours by TLC. The pH was lowered to 9 by the addition of HCl (4 N). DMF was evaporated via high vacuum rotovap, and syrup was taken up in ethylacetate (600 ml) and washed with sodium bicarbonate (3 X 300 mL). The aqueous layer was washed with ethylacetate (3 X 400 ml), and all organic extracts were combined, dried with sodium sulfate, and solvents removed to give 99% product.

<u>2-(5-Methoxy-2-methyl-1*H*-indol-3-yl)-ethylamine (Compound **268**)</u>. Compound **303** (273 mg, 0.7 mmol) was dissolved in freshly distilled THF (30 ml) and cooled to 0°C. Slow addition of a 1 M solution of LAH (0.85 ml, 0.85 mmol) was made with vigorous gaseous evolution noted. The reaction was stirred at room temperature (RT) for 6 days (144 hours), after which time it was poured slowly onto ice water and diluted with ether (150 ml). The aqueous layer was washed with ether (2 X 150 mL) and all ether extracts were combined and acidified with HCl (1 N, 3 X 150 mL). The acid extracts were treated with 4 N NaOH until pH 10 and the products were extracted into ether (3 X150 mL), dried, and concentrated to give selectively 1-(4-

bromobenzyl)-5-methoxy-2-methyl-3-indolethylamine in 55% yield. No 1-benzyl-5-methoxy-2-methyl-3-indolethylamine was detected.

[2-(5-Methoxy-2-methyl-1*H*-indol-3-yl)-ethyl]-carbamic acid *tert*-butyl ester (Compound **277**). Compound **268** (50 mg, 0.25 mmol) was stirred while dicarbonate (64 mg, 0.29 mmol) in DMF (50 μL) was added at 23°C. The reaction stirred for 18 hours and was judged complete by TLC. The reaction was concentrated to a syrup and dissolved in EtOAc (5 mL), washed with saturated sodium bicarbonate (2 x 2 mL), dried with sodium sulfate, and concentrated to give product (74 mg; 99%) which was used immediately in the next step.

{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-carbamic acid *tert*-butyl ester (Compound **278**). NaH (7 mg, 0.29 mmol) was added dropwise to a solution of Compound **277** (74 mg, 0.25 mmol) in DMF (10 mL) at 0°C. The reaction mixture was stirred for 20 minutes at 0°C at which time bromobenzyl bromide (72 mg, 0.29 mmol) was added. The reaction stirred overnight and was diluted carefully with water, extracted with ether (2 x 10 mL) and washed with water (2 x 5 mL), dried with sodium sulfate, concentrated, and purified on silica (EtOAc 10% in hexanes) to give a yellow solid (20 mg, 17%)

2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine (Compound **279**). HCl gas ($HCl_{(g)}$) was gently bubbled through a 1 mL solution of Compound **278** in CH_2Cl_2 in a 2 mL vial for 1 hour. The reaction was diluted with water and neutralized with 1 N NaOH added dropwise until pH = 9. The product was extracted with $CHCl_3$ (3 x 3 mL) and dried with sodium sulfate to give a yellow oil (13 mg, 84%)

Example 8

Synthesis of Diamide Derivatives of Indomethacin

Diamide derivatives of indomethacin were synthesized following the general scheme outlined in Figure 17.

(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-carbamic acid *tert*-butyl ester (Compound **365**). In an

oven dried round bottomed flask equipped with a magnetic stir bar and a rubber septum were placed indomethacin (1 eq) HOBt (1.1 eq) and (1-[(3-dimethylamino)propyl]-3-ethylcarbodiimide (1.1 eq) in anhydrous CH_2Cl_2. To this was added a solution of the BOC-protected diamine in CH_2Cl_2. Stirring was continued for 18 hours. The reaction mixture was then quenched by pouring the mixture into a separatory funnel containing aqueous saturated sodium bicarbonate followed by H_2O. The organic layer was collected and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure to give a yellow solid. Purification was performed by flash column chromatography (silica gel, 50% EtOAc in Hexane) to give a white powder (7.7g, 60%). ^1H NMR ($CDCl_3$) δ 7.70 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 6.91-6.88 (m, 2H), 6.69 (dd, J = 2.1, 9.1 Hz, 2 H), 6.29 (s, 1H), 3.82 (s, 3H), 3.63 (s, 2H), 3.35-3.29 (m, 2H), 3.21-3.16 (m 2H), 2.38 (s, 3H), 1.35 (s, 9H); ESI 500 (MH^+)

<u>*N*-(2-Amino-ethyl)-2-[1-(4-chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-acetamide (Compound **377**)</u>. The appropriate indo-BOC-aminoamide (1 eq) was dissolved in CH_2Cl_2 in a three neck round bottomed flask fitted with a reflux condenser in the center. A septum was placed in one opening while a second septum with a hole bored into it and containing a glass pasture pipette. The pipette was connected to the HCl gas cylinder via a TEFLON® tube. Gentle bubbling of the gas was maintained for 0.5 hours during which time the reaction develops a precipitate. TLC confirmed the consumption of starting material. The crude reaction was then concentrated *in vacuo* to give a solid (722 mg, 99%), which was used without further purification. ^1H NMR ($CDCl_3$) δ 7.66 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 6.91-6.88 (m, 3H), 6.69 (dd, J = 2.2, 9.0 Hz, 2 H), 6.29 (s, 1H), 3.82 (s, 3H), 3.65 (s, 2H), 3.28-3.23 (m, 2H), 2.75 (s, 2H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6, 168.7, 156.6, 139.9, 136.6, 134.0, 131.6, 131.3, 130.8, 129.6, 115.5, 113.4, 112.6, 101.2, 56.1, 42.6, 41.6, 32.7, 28.7, 13.7; ESI 400 (MH^+).

N-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-acetylamino}-ethyl)-4-dimethylamino-benzamide (Compound **354**). Compound **351** (210 mg, 0.5 mmol), dimethylaminobenzoic acid (264 mg, 1.5 mmol), EDCI (304 mg, 1.5 mmol), HOBt (215 mg, 1.5 mmol) and DIPEA (87 μL, 1.5 mmol) were dissolved in DMF (dry, 15 mL) and allowed to stir 18 hours. The reaction was quenched with saturated sodium bicarbonate (30 ml) and diluted with $CHCl_3$ (30 mL). The organic layers were combined, and concentrated and purified on silica gel (25% EtOAc in hexanes) to give a white solid (118 mg, 43%); ^{1}H NMR (MeOH-d_4) δ 7.68 (d, J = 8.5 Hz, 2H), 7.48 (d, J = 8.9 Hz, 2H), 7.42 (d, J = 8.5 Hz, 2H), 6.84 (d, J = 9.2 Hz, 2H), 6.73 (s, 1H), 6.65 (dd, J = 2.3, 9.0 Hz, 1H), 6.57 (d, J = 8.9 Hz, 1H), 3.75 (s, 3H), 3.61 (s, 2H), 3.44 (s, 4H), 2.34 (s, 3H); ^{13}C NMR (MeOH-d_4) δ 171.9, 168.8, 156.6, 139.5, 138.0, 137.0, 134.2, 131.7, 131.4, 130.7, 129.5, 128.8, 121.7, 115.6, 113.0, 112.5, 111.4, 101.1, 56.1, 41.3, 40.5, 32.5, 13.7; ESI-CID 547 (MH^+) m/z 382, 148.

N-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-acetylamino}-ethyl)-4-fluoro-benzamide (Compound **355**). Compound **351** (210 mg, 0.5 mmol), fluorobenzoic acid (210 mg, 1.5 mmol), EDCI (304 mg, 1.5 mmol), HOBt (215 mg, 1.5 mmol) and DIPEA(87 μL, 1.5 mmol) were dissolved in DMF (dry, 15 mL) and allowed to stir 18 hours. The reaction was quenched with saturated sodium bicarbonate (30 ml) and diluted with $CHCl_3$ (30 mL). The organic layers were combined and concentrated and purified on silica gel (25% EtOAc in hexanes) to give a white solid (72 mg, 30%); ^{1}H NMR ($CDCl_3$) δ 7.68 (d J = 8.6 Hz, 2H), 7.65-7.62 (m, 1H). 7.57, (d, J = 8.6 Hz, 1H), 7.45 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.6 Hz, 1H), 7.23-7.10 (m, 1H), 7.04 (t, J = 8.6 Hz, 2H), 6.86-6.82 (m, 2H), 6.65 (dd, J = 2.4, 9.1 Hz, 1H), 6.56-6.50 (m, 1H), 3.74 (s, 3H), 3.63 (s, 2H), 3.50-3.40 (m, 4H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.5, 168.7, 167.4, 156.6, 139.9, 137.0, 134.0, 131.6, 130.7, 129.7, 129.6, 129.2, 128.8, 116.1, 115.8, 115.6, 112.7, 112.4, 101.2, 56.01, 41.5, 40.7, 32.5, 13.7; ESI-CID 522 (MH^+), m/z 312, 245, 174.

N-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-acetylamino}-ethyl)-4-trimethylamoniumtrifluoromethanesulfonyl-benzamide (Compound **361**). Compound **354** (31.2 mg, 0.057 mmol) was dissolved in CH_2Cl_2 (dry, 20 mL) and methoxy trifluoromethane sulfonate (7.5 μL, 0.068 mmol) was added dropwise. The reaction was stirred for 18 hours, after which another aliquot of the triflate (20 μL) was added. The reaction was stirred for another 18 hours, at which time ether was added (5 mL) to produce a slight precipitate. Distilled water (20 mL) was added to dissolve the precipitate and the aqueous layer was collected and concentrated to give a green oil (25 mg, 62%); ^{1}H NMR (MeOH-d_4) δ 7.78 (d, J = 9.2 Hz, 2H), 7.72 (d, J = 9.1 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 6.91-6.86 (m, 2H), 6.53 (dd, J = 2.3, 9.1 Hz, 1H), 3.66 (s, 3H), 3.59 (s, 9H), 3.42-3.38 (m, 4H), 2.15 (s, 3H); ^{13}C NMR (MeOH-d_4) δ ESI-CID 561 (MH^+), *m/z* 312, 148.

N-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1H-indol-3-yl]-acetylamino}-ethyl)-4-hydroxy-benzamide (Compound **380**). Compound **377** (106 mg, 0.27 mmol), EDCI (76 mg, 0.40 mmol), DIPEA(70 μL, 0.40 mmol) *p*-hydroxybenzoic acid (55 mg, 0.40 mmol) and HOBt (54 mg, 0.40 mmol) were dissolved in DMF (dry, 20 mL) and allowed to stir for 36 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate (3 x 30 mL) and diluted with EtOAc (30 mL). The organic layer was concentrated *in vacuo* and purified on silica (EtOAc 80% in hexanes) to give a white solid, which was recrystallized from EtOAc (52 mg, 38%); ^{1}H NMR 400 MHz (DMSO-d_6) δ 9.92 (s, 1H), 8.18 (t, J = 5.1 Hz, 1H), 8.07 (t, J = 5.0 Hz, 1H), 7.67 (d, J = 8.6 Hz, 2H), 7.64-7.61 (m, 4H), 7.08 (d, J = 2.3 Hz, 1H), 6.93 (d, J = 9.0 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 6.69 (dd, J = 2.4, 9.0 Hz, 1H), 3.73 (s, 3H), 3.49 (s, 2H), 2.05-1.77 (m, 4H), 2.49 (s, 3H); ^{13}C NMR 400 MHz (DMSO-d_6) δ 170.1, 168.2, 166.6, 160.5, 155.9, 137.9, 136.0, 135.6, 135.0, 134.6, 131.5, 131.3, 130.7, 129.4, 115.1, 114.9, 114.5, 111.6, 102.1, 55.8, 31.6, 13.7; ESI 520 (MH^+).

<u>*N*-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-4-iodo-benzamide (Compound **381**)</u>. Compound **377** (109 mg, 0.27 mmol), EDCI (78 mg, 0.41 mmol), DIPEA (71 μL, 0.41 mmol), *p*-iodobenzoic acid (102 mg, 0.41 mmol), and HOBt (55 mg, 0.41 mmol) were dissolved in DMF (dry, 20 mL) and allowed to stir for 36 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate (3 x 30 mL) and diluted with EtOAc (30 mL). The organic layer was concentrated *in vacuo* and purified on silica (EtOAc 80% in hexanes) to give a white solid, which was recrystallized from EtOAc (88.4 mg, 52%); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.64 (m, 1H), 7.25 (m, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 6.77 (d *J* = 8.3 Hz, 2H), 6.67 (d, *J* = 8.1 Hz, 2H), 6.24 (s, 1H), 6.08 (d, *J* = 9.0 Hz, 1H), 5.83 (dd, *J* = 2.2, 8.9 Hz, 1H), 2.88 (s, 3H), 2.65 (s, 2H), 2.42-2.38 (m, 4H), 1.34 (s, 3H); ^{13}C NMR 400 MHz (DMSO-d_6) δ 170.2, 168.2, 166.2, 155.9, 137.9, 137.4, 135.6, 134.6, 134.2, 131.5, 131.2, 130.7, 129.5, 129.4, 114.9, 114.5, 111.6, 102.2, 99.1, 55.7, 31.6, 13.7; ESI 630 (MH$^+$).

<u>*N*-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-4-nitro-benzamide (Compound **382**)</u>. Compound **377** (117 mg, 0.29 mmol), EDCI (84 mg, 0.44 mmol), DIPEA (77 μL, 0.44 mmol), *p*-nitrobenzoic acid (74 mg, 0.44 mmol), and HOBt (59 mg, 0.44 mmol) were dissolved in DMF (dry, 20 mL) and allowed to stir for 36 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate (3 x 30 mL) and diluted with EtOAc (30 mL). The organic layer was concentrated *in vacuo* and purified on silica (EtOAc 80% in hexanes) to give a white solid, which was recrystallized from EtOAc (107 mg, 67%); ^{1}H NMR 400 MHz (DMSO-d_6) δ 7.86 (d, *J* = 5.4 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.24 (d, *J* = 5.2 Hz, 1H), 7.09 (d, *J* = 6.9 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.76 (d, *J* = 8.6 Hz, 2H), 6.23 (d, *J* = 2.4 Hz, 1H), 6.05 (d, *J* = 9.0 Hz, 1H), 5.81 (dd, *J* = 2.5, 9.0 Hz, 1H), 2.87 (s, 3H), 2.65 (s, 2H), 2.43 (m, 4H), 1.33 (s, 3H) ; ^{13}C NMR (DMSO-d_6) δ 170.2, 168.2, 165.2, 155.9, 149.2, 140.4,

137.9, 135.6, 134.6, 131.5, 131.2, 130.7, 129.4, 129.0, 123.7, 114.9, 114.5, 102.2, 55.7, 31.6, 13.7; ESI-CID 549 (MH^+).

Toluene-4-sulfonic acid 4-(2-{2-[1-(4-chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethylcarbamoyl)-phenyl ester (Compound **387**). Compound **380** (14.5 mg, 0.028 mmol) was dissolved in DMF (2 mL) with pyridine (2 drops). Tosyl chloride (6 mg, 0.031 mmol) was added and the reaction vessel was purged with argon and stirred at room temperature for 15 hours. The reaction was quenched with saturated sodium bicarbonate (2 x 10 mL) and extracted into CH_2Cl_2 (2 x 20 mL). The combined organic solution was washed with water (2 x 20 mL), dried with sodium sulfate, concentrated, and purified on silica (EtOAc 50% in hexanes) to give a yellow solid (6.3 mg, 33%); ^1H NMR (MeOH-d_4) δ 7.60 (d, J = 8.1 Hz, 4H), 7.48 – 7.43 (m, 4H), 7.30 (d, J = 8.0 Hz, 2H), 6.88 – 6.85(m, 3H), 6.78 (d, J = 9.0 Hz, 1H), 6.50 (dd, J = 9.0, 2.4 Hz, 1H), 3.65 (s, 3H), 3.51 (s, 2H), 3.23 (m, 4H), 2.34 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (MeOH-d_4) δ 174.4, 170.4, 169.5, 158.0, 153.7, 147.9, 140.5, 137.7, 136.1, 134.7, 133.8, 132.8, 132.7, 132.5, 131.6, 130.6, 130.4, 130.1, 123.8, 116.4, 114.9, 113.1, 102.7, 56.5, 41.2, 41.0, 32.8, 22.1, 14.0;

Example 9

Synthesis of Amide Derivatives of Indomethacin

Amide derivatives of indomethacin were synthesized using the general scheme outlined in Figure 18.

N-(4-Fluoro-phenyl)-2-(5-methoxy-2-methyl-1*H*-indol-3-yl)-acetamide (Compound **375**). Method A. To a solution of 5-methoxy-2-methyl-1*H*-indolacetic acid (1g, 4.6 mmol) in dry CH_2Cl_2 (30 mL) was added DMAP (0.83 g, 6.8 mmol) and EDCI (1.3 g, 6.8 mmol) followed by 4-fluoroaniline (0.65 mL, 6.8 mmol). The reaction was allowed to stir for 18 hours at 23°C. The mixture was diluted with water (30 mL) and extracted with EtOAc (2 x 30 mL). The combined organic extracts were washed with water (2 x 30 mL), dried with sodium sulfate, concentrated, and purified on silica (20% EtOAc in hexanes) to give a white powder (433 mg, 30%).

Method B. Compound **360** (341 mg, 0.76 mmol; *see* Figure 18) was dissolved in dry DMF (20 mL) and 10 N NaOH (513 μL) was added portionwise over 3 hours. The reaction was judged complete by TLC and quenched with water (100 mL) and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with water (2 x 30 mL) and dried ($MgSO_4$) to give a white powder (203 mg, 86%), which was used without further purification. ^1H NMR 400 MHz ($CDCl_3$) δ 8.04 (s, 1H), 7.37 (s, 1H), 7.31-7.24 (m, 3H), 6.95-6.90 (m, 3H), 6.83 (dd, *J* = 2.4, 8.7 Hz, 1H), 3.81 (s, 3H), 3.78 (s, 2H), 2.42 (s, 3H)

2-[1-(4-Chloro-2-fluoro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-(4-fluoro-phenyl)-acetamide (Compound **360**). Compound **375** (57 mg, 0.18 mmol) was dissolved in dry DMF (10 mL) and cooled to 0°C. NaH (8.7 mg, 0.36 mmol) was added portionwise and the reaction was stirred for 20 minutes. To the reaction was added 2-fluoro-4-chloro-benzoyl chloride (70 mg, 0.36 mmol). The mixture was allowed to stir at 23°C for 17 hours at which time TLC showed ~50% conversion of starting material. Another 70 mg of the benzoyl chloride followed by 15 mg NaH was added to the reaction and allowed to stir for an additional 18 hours. The reaction was poured carefully onto ice water (20 mL) and extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with 10% HCl (2 x 10 mL), dried with sodium sulfate, purified on silica (10% EtOAc in hexanes) to give yellow solid (28 mg, 33%); ^1H NMR ($CDCl_3$) δ 7.94 (t, *J* = 9.0 Hz, 1H), 7.59 (t, *J* = 8.1 Hz, 1H), 7.34-7.30 (m, 3H), 7.30-7.17 (m, 2H), 6.96 (d, *J* = 8.9 Hz, 1H), 6.91 (d, *J* = 1.2 Hz, 1H), 6.76 (dd, *J* = 2.5, 9.0 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 2H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 169.0, 163.0, 156.4, 135.7, 135.2, 133.7, 131.69, 130.1, 126.2, 125.3, 121.3, 120.5, 118.1, 117.8, 117.5, 115.8, 115.5, 115.1, 111.9, 102.7, 55.8, 32.2, 13.7; ESI 491 (MNa^+).

4-Chloro-2-nitro-benzoyl chloride (Compound **384**). A mixture of 4-Chloro-2-nitro-benzoic acid (2g, 9.9 mmol) and $SOCl_2$ (8.5 mL, 114.8 mmol) and DMF(66 μL) was stirred at 26°C for 4 hours. When evolution of HCl subsided the temperature was raised to 65°C with stirring for 1 hours. After

removal of excess $SOCl_2$ by vacuum distillation, the residue was dissolved in 1,2 dichloromethane (2 mL) and evaporated. The residue was dissolved in 10 mL of 1,2 dichloromethane and treated twice with decolorizing charcoal and filtered to give the final product in quantitative yield which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.95 (s, 1H), 7.74 (s, 2H).

2-[1-(4-Chloro-2-nitro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-(4-fluoro-phenyl)-acetamide (Compound **385**). Compound **375** (100 mg, 0.32 mmol) was dissolved in DMF (dry, 5 mL) and cooled to 0°C. NaH (7.7 mg, 0.32 mmol) was added portionwise and the reaction was allowed to stir for 20 minutes. A clear to yellow color change was noted. Compound **384** (100 μL, 0.48 mmol) was added dropwise with an immediate color change to orange. The reaction stirred for 18 hours and was allowed to warm to room temperature. The reaction was diluted in CH_2CL_2 (30 mL) and quenched with 10% HCl (30 mL) solution. The organic layer was concentrated and purified on silica gel (EtOAc, 20% in hexanes) to give a brown syrup (51 mg, 32%); ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 1.9 Hz, 1H), 7.81 (dd J = 1.9, 8.2 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.40-7.36 (m, 2H), 7.28 (s, 1H), 6.97-6.84 (m, 4H), 6.68 (dd, J = 2.3, 9.0 Hz, 1H), 3.79 (s, 3H), 3.74 (s, 2H), 2.39 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 168.5, 164.4, 156.6, 146.8, 136.5, 135.8, 135.4, 135.2, 131.9, 131.2, 131.0, 130.1, 125.8, 121.4, 121.3, 116.0, 115.8, 115.5, 112.0, 103.0, 55.8, 32.5, 14.0; ESI 471 (MH^+).

2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethylamine (Compound **388**). Compound **277** (136 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (dry, 6 mL) and $HCl_{(g)}$ was bubbled gently through mixture until TLC indicated complete consumption of starting material. Saturated sodium bicarbonate (15 mL) was slowly added to neutralize mixture which was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic solution was washed with water (2 x 20 mL), dried with sodium sulfate, and concentrated to give the product in quantitative yield (107 mg, 100%), which was used without further purification.

<u>*N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-nitro-benzamide (Compound **389**)</u>. Compound **388** (42 mg, 0.11 mmol), EDCI (25 mg, 0.13 mmol), DIPEA (23 μL, 0.13 mmol) *p*-nitrobenzoic acid (22 mg, 0.13 mmol), and HOBt (18 mg, 0.13 mmol) were dissolved in DMF (dry, 5 mL) and allowed to stir for 18 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate (2 x 10 mL) and extracted with EtOAc (2 x 20 mL). The combined organic solution was washed with water (2 x 20 mL), dried with sodium sulfate, concentrated, and purified on silica (EtOAc 50% in hexanes) to give a yellow solid (11 mg, 20%); ^{1}H NMR ($CDCl_3$) δ 8.18 (d, *J* = 8.8 Hz, 2H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 7.00 (d, *J* = 2.3 Hz, 1H), 6.81-6.76 (m, 3H), 6.20 (s, 1H), 5.20 (s, 2H), 3.78 (s, 3H), 3.72-3.71 (m, 2H), 3.08-3.06 (m, 2H), 2.24 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 154.7, 137.3, 132.3, 128.3, 128.0, 124.2, 111.4, 110.4, 100.7, 56.31, 46.61, 24.53, 10.76; ESI-CID 522 (MH^+).

<u>*N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-fluoro-benzamide (Compound **390**)</u>. Compound **388** (42 mg, 0.11 mmol), EDCI (25 mg, 0.13 mmol), DIPEA (23 μL, 0.13 mmol) *p*-fluorobenzoic acid (18 mg, 0.13 mmol), and HOBt (18 mg, 0.13 mmol) were dissolved in DMF (dry, 5 mL) and allowed to stir for 18 hours at room temperature. The reaction was quenched with saturated sodium bicarbonate (2 x 10 mL) and extracted with EtOAc (2 x 20 mL). The combined organic solution was washed with water (2 x 20 mL), dried with sodium sulfate, concentrated, and purified on silica (EtOAc 50% in hexanes) to give a yellow solid (30 mg, 56%); ^{1}H NMR ($CDCl_3$) δ δ 8.11-8.08 (m, 1H), 7.61-7.56 (m, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.16-6.98 (m, 5H), 6.77 (d, *J* = 8.5 Hz, 2H), 5.19 (s, 2H), 3.76 (s, 3H), 3.69-3.67 (m, 2H), 3.06-3.01 (m, 2H), 2.23 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 154.7, 137.4, 132.3, 129.5, 129.4, 128.6, 128.1, 116.1, 115.9, 111.4, 110.3, 108.9, 100.7, 56.3, 51.3, 46.6, 41.0, 31.3, 24.7, 10.7; ESI-CID 595 (MH^+), *m/z* 356.1, 194.5.

<u>[1-(4-Chloro-2-nitro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetic acid (Compound **391**)</u>. 5-methoxy-2-methyl-1*H*-indol-3-yl]-acetic acid (315

mg, 1.43 mmol) was dissolved in DMF (dry, 5 mL) and cooled to 0°C. NaH (69 mg, 2.88 mmol) was added portionwise and the reaction was allowed to stir for 20 minutes. 4-Chloro-2-fluoro-benzoyl chloride (275 μL, 2.15 mmol) was added. The reaction was allowed to stir for 18 hours and allowed to warm to room temperature. The reaction was quenched with 10 % HCl (50 mL) and extracted in CH_2CL_2 (50 mL). The combined organic solution was washed with water (2 x 20 mL), dried with sodium sulfate, concentrated, and purified on silica gel (EtOAc, 25% in hexanes) to give a brown solid.

Example 10

Radiolabeling of Indomethacin Derivatives

The production of ^{18}F and the exchange chemistry is shown in Scheme 4 (*see* Figure 19). The fluorine-18 anion was prepared from ^{18}O-water using the 12 MeV cyclotron at the Vanderbilt Medical Center Nuclear PET facility (Vanderbilt University, Nashville, Tennessee, United States of America). The fluorine-18 anion was then trapped onto an anion exchange column, and eluted with potassium carbonate to give $K^{18}F$. The ion pair was delivered to the reaction vessel and complexed with $KRYPTOFIX_{2,2,2}^{®}$ to generate the [$KRYPTOFIX_{2,2,2}^{®}$ – K^+] [F^-] ion complex. Upon drying the salt down, substrate (dissolved in 5 mL acetonitrile) was delivered to the reaction vessel and the temperature was brought to 85°C. The reaction was allowed to stand for 30 minutes and then removed from the exchange apparatus for workup and radio-TLC quantification.

Materials and Methods for Examples 11-12

Enzymology. Arachidonic acid was purchased from Nu Chek Prep (Elysian, Minnesota, United States of America). [1-^{14}C]Arachidonic acid (~55-57 mCi/mmol) was purchased from NEN Dupont (Boston, Massachusetts, United States of America) or American Radiolabeled Chemicals, Inc. (St. Louis, Missouri, United States of America). COX-1 was purified from ovine seminal vesicles (Oxford Biomedical Research, Inc., Oxford, Michigan, United States of America) as described in Marnett *et al.*, 1984. The specific activity of the protein was 20 (μM O_2/min)/mg, and the

percentage of holoprotein was 13.5%. ApoCOX-1 was prepared by reconstitution by the addition of hematin to the assay mixtures as described in Odenwaller *et al.*, 1990. Apoenzyme was reconstituted by the addition of hematin to the assay mixtures. Human COX-2 was expressed in Sf9 insect cells by means of the pVL 1393 expression vector (BD Biosciences Pharmingen, San Diego, California, United States of America) and purified by ion exchange and gel filtration chromatography. All of the purified proteins were shown by densitometric scanning of a 7.5% SDS-PAGE gel to be >80% pure.

Time- and Concentration-Dependent Inhibition of Ovine COX-1 and Human COX-2 Using Thin Layer Chromatography (TLC) Assay. Cyclooxygenase activity of ovine COX-1 (44 nM) or human COX-2 (66 nM) was assayed by TLC. Reaction mixtures of 200 μL consisted of hematin-reconstituted protein in 100 mM Tris-HCl, pH 8.0, 500 μM phenol, and [1-^{14}C]arachidonic acid (50 μM) for 30 seconds at 37°C. Reactions were terminated by solvent extraction in Et_2O/CH_3OH/1 M citrate, pH 4.0 (30:4:1). The phases were separated by centrifugation at 2000*g* for 2 minutes and the organic phase was spotted on a TLC plate (J. T. Baker, Phillipsburg, New Jersey, United States of America). The plate was developed in EtOAc/CH_2CL_2/glacial AcOH (75:25:1)) at 4°C. Radiolabeled prostanoid products observed at different inhibitor concentrations was divided by the percentage of products observed for protein samples preincubated for the same time with DMSO.

Inhibition of COX-2 Activity in Activated RAW264.7. Protocols for COX-2 inhibition in RAW264.7 cells have been previously described (Kalgutkar *et al.*, 1998b). Briefly, cells (6.2 X 10^6 cells/T25 flask) were activated with lipopolysaccharide (1 μg/mL) and γ-interferon (10 U/mL) in serum-free DMEM for 7 hours and then treated with inhibitor (0-2 μM) for 30 minutes at 37°C. Exogenous arachidonate metabolism was determined by adding [1-^{14}C]-arachidonate acid (20 μM) for 15 minutes at 37°C. IC_{50} values are the average of two independent determinations.

Example 11

Selective COX-2 Inhibition in Purified Enzyme

IC_{50} values for the inhibition of purified human COX-2 or ovine COX-1 by test compounds were determined by thin layer chromatography (TLC) radiography. Hematin-reconstituted COX-2 (66 nM) or COX-1 (44 nM) in 100 mM Tris-HCl, pH 8.0 containing 500 μM phenol was treated with several concentrations of inhibitors (0-2850 nM) at 25°C for 20 minutes. The cyclooxygenase reaction was initiated by the addition of [1-^{14}C]-arachidonic acid (50 μM) at 37°C for 30 seconds. As indicated in Tables 1-3 below, the fluorinated standards Compounds **355**, **360**, and **389** displayed potent and selective inhibition of COX-2 over COX-1 with IC_{50} values in the 50-100 nM range.

Example 12

Selective COX-2 inhibition in RAW264.7 Murine Macrophages

The ability of the fluorinated amide analogs of indomethacin to inhibit COX-2 in intact cells was assayed in RAW264.7 macrophages in which COX-2 activity was induced by pathologic stimuli. The macrophages were exposed to lipopolysaccharide and γ-interferon to induce COX-2 and then treated with several concentrations of Compound **355**. The IC_{50} value for inhibition of prostaglandin D2 production by Compound **355** was 500 nM.

Discussion of Examples 7-12

Three representative COX-2 selective indomethacin analog precursors for positron emitting tomography (PET) were designed and prepared to investigate the feasibility of a COX-2 selective tumor imaging agent. A fluorinated amide, an indolyl amide, and a diamide analog of indomethacin have been shown to exhibit potent and selective activity against COX-2 *in vitro* over COX-1 in assays (COX-1 IC_{50} > 60 μm for all, COX-2 IC_{50} = 50-100 nm). The synthesis of 2-[1-(4-Chloro-2-fluoro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-(4-fluoro-phenyl)-acetamide (Compound **360**), *N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-fluoro-benzamide (Compound **390**) and *N*-(2-{2-[1-(4-Chloro-

benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-4-fluoro-benzamide (Compound **382**) were all carried out using EDCI amide coupling to give 33%, 43% and 56% yields respectively from the appropriate amide precursors. The nitro benzamide analogs were prepared similarly to give 2-[1-(4-Chloro-2-nitro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-*N*-(4-fluoro-phenyl)-acetamide (Compound **360**), 32%; *N*-(2-{2-[1-(4-Chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-ethyl)-4-nitro-benzamide (Compound **382**), 67%; and *N*-{2-[1-(4-Bromo-benzyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-ethyl}-4-fluoro-benzamide (Compound **390**), 56%. The nitro or tosyl compounds can be exchanged by nucleophilic aromatic substitution to generate ^{18}F PET agents.

<u>Indolyl Amides of Indomethacin</u>

The imaging agents in indoyl amide series utilized commercially available indomethacin which was transformed in 7 steps to either the fluoro-standard, Compound **389**, or the PET precursor, Compound **390,** using Scheme 1 depicted in Figure 16. The development of this synthetic pathway was the result of several pathways tested. Indomethacin was first converted to the acetamide, Compound **301**, followed by debenzoylation of the *p*-chlorobenzoyl group to give Compound **303**. The 3rd step involved the protection of the free amine using BOC anhydride so that selective benzylation of the indole nitrogen could be accomplished. Subsequent $HCl_{(g)}$ deprotection of the BOC group followed by amidation using the appropriate *p*-substituted benzoic acid gave the PET precursor or fluorinated standard, Compounds **390** and **389**, respectively, in good overall yield.

<u>Diamide Derivatives of Indomethacin</u>

The synthesis of diamide indomethacin imaging agents required the selective amidation of only one of the two available amino groups present in the diamine tether. Dimer prevention was accomplished by the use of the mono *tert*-butoxycarbonyl (BOC) protected diamine. Treatment of indomethacin with mono BOC-ethylenediamine in the presence of ethyl-1-[3-(dimethylamino)propyl]-3-ethylcarbodiamide (EDCI) afforded the desired

products in good yield using Scheme 2 (*see* Figure 17). 1-hydroxybenzotriazole hydrate (HOBt) was employed, as it perturbed the generation of the stable, undesired N-acylurea byproduct. Deprotection of the BOC group was cleanly and efficiently accomplished by bubbling HCl gas through a solution of methylene chloride and the amino amide. Generation of the benzamide derivatives Compounds **354, 355,** and **380-382**, was accomplished with EDCI coupling in the presence of HOBt and DIPEA in DMF.

Amide Derivatives of Indomethacin

The amide series can be synthesized from many routes, depending on the availability of starting materials. Preparation of the amide Compound **385** was accomplished by convenient $HCl_{(g)}$ debenzoylation of Compound **360** to give Compound **375** followed by benzoylation using the corresponding acid chloride according to Scheme 3 (*see* Figure 18).

Alternatively, Compound **375** was prepared from the commercially available indole acetic acid via EDCI coupling. *o*-Nitro benzaldehydes have been shown to undergo PET exchange (*see* Ekaeva *et al.*, 1995), so the exchangable group was placed ortho to the amide withdrawing group on the benzoyl chloride functionality. The 4-chloro-2-nitro benzoylchloride (Compound **384**) was prepared by stirring the benzoic acid starting material with thionyl chloride in DMF initially at room temperature until all HCl generation subsided followed by reflux for one hour. Benzoylation of Compound **384** to the indole nitrogen was accomplished by treatment of the indole with NaH for 10 minutes before Compound **384** was added.

In some embodiments, disclosed herein are reverse amides of indomethacin. The reverse amide series is different from those of the indomethacin series due to the placement of the amide bond. This amide "reversal" design was created to address the metabolic and hydrolytic instability associated with the conventional indomethacin analogs. Furthermore, amide bond hydrolysis in these compounds following *in vivo* administration in preclinical species will not generate indomethacin.

The diamide series was developed to address the feasibility of tethering bulky functional groups onto indomethacin to create a "dual function" inhibitor. The use of a long aliphatic chain allows the indomethacin functionality to fully insert into the binding pocket of COX-2 while the bulky secondary amide functional group resided in the more spacious lobby of the enzyme. Incorporating the diamine tether between indomethacin and *p*-fluorobenzamide aided this interaction. Extensive testing of Compound **355** has shown that this compound is selective and potent against COX-2 in free enzyme as well as intact cells.

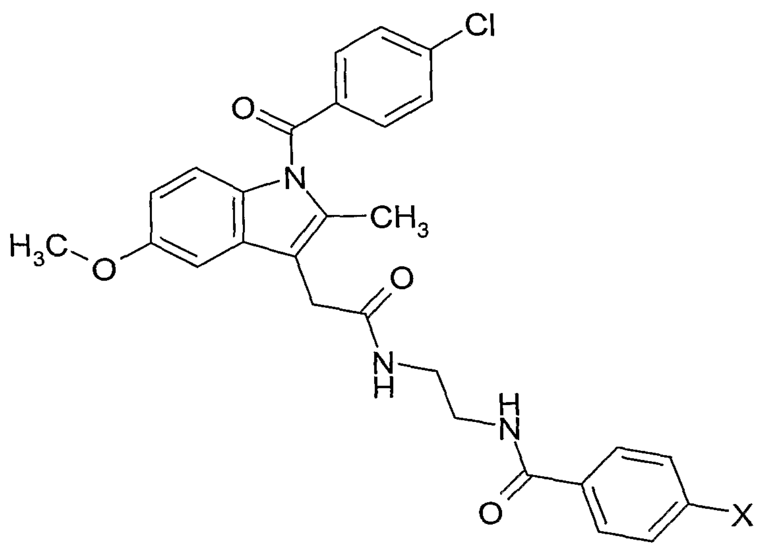
Lastly, the amide series was developed in order to place the exchangeable group in the indomethacin core. This allows a large array of amides or esters to be prepared to address the issues of selectivity, potency, and half-life. The synthesis of a large series of derivatives could be accomplished by first benzoylating 5-methoxy-2-methyl indole with the appropriate PET sensitive acid chloride followed by amidation using a variety of amines.

An improved synthesis of the reverse amide intermediate has been accomplished to afford efficient reduction of the amine and selective benzylation at the indole nitrogen to give the key intermediate in gram scale quantities. The diamide series has been fully utilized for PET with the discovery that Compound **355** is a potent and specific inhibitor of COX-2 both in free enzyme as well as intact cells. The amide series also shows promise.

Tables 1-3 show several series of potential PET precursors as well as the ^{19}F standards. Also provided are IC_{50} values for certain of the derivatives for COX-1 and COX-2.

Table 1

Diamide Series Indomethacin Derivatives

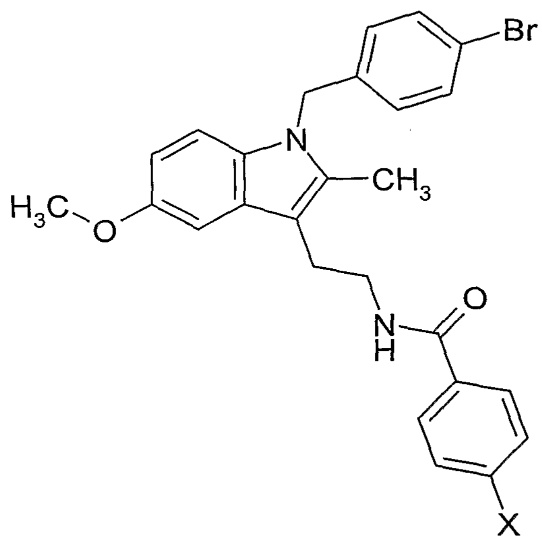


Compound No.	X
355	F
361	NMe_3^+
381	I
382	NO_2
387	OTs

355: COX-1 IC_{50} > 60 μM; COX-2 IC_{50} 103 nM

Table 2

Reverse Amide Series Indomethacin Derivatives

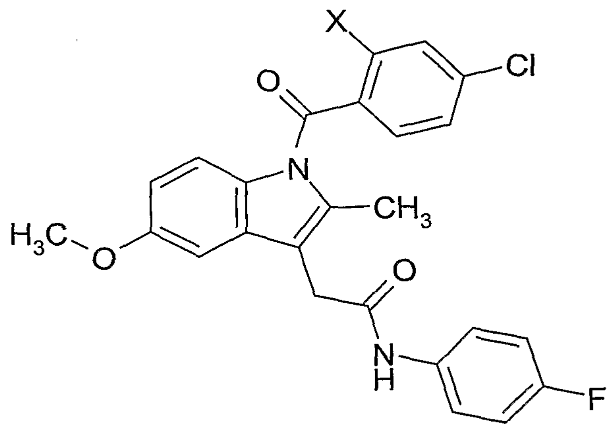


Compound No.	X
389	F
390	NO_2
-	I
-	OTs
-	NMe_3^+

389: COX-1 IC_{50} > 60 μM; COX-2 IC_{50} 53 nM

Table 3

Amide Series Indomethacin Derivatives



Compound No.	X	R
360	F	NH-C_6H_4-F
385	NO_2	NH-C_6H_4-F
391	F	OH
-	I	NH-C_6H_4-F
-	OTs	NH-C_6H_4-F
-	NMe_3^+	NH-C_6H_4-F

360: COX-1 IC_{50} > 60 μM; COX-2 IC_{50} 100 nM

Example 13

Pharmacokinetics and Metabolism

The *in vivo* pharmacokinetics and pharmacodynamics of the indomethacin derivatives are of interest in the design of an imaging agent, in that compounds that exhibit lengthy half-lives are more likely to reach target tissues. Detailed metabolic studies have been performed on three compounds, shown in Figure 13. All three compounds are highly potent and selective COX-2 inhibitors, as indicated by IC_{50} values for the purified enzyme of 0.060 μM, 0.060 μM, and 0.052 μM for Compounds **16**, **17**, and

18 (Figure 11), respectively. All three compounds demonstrated IC_{50} values for COX-1 of > 66 μM.

Preliminary metabolic studies were conducted using isolated liver microsome preparations. Compound **16** was rapidly metabolized by rat, human, and mouse liver microsomes (0.125 mg/mL protein), with half-lives of 11 minutes, 21 minutes, and 51 minutes, respectively. Four metabolites were identified that arise by hydroxylation of the ethylene side chain and demethylation of the 5-methoxy group on the indole ring. The latter is a minor pathway of metabolism. Studies using specific inhibitors of cytochrome P450 isoforms, and purified recombinant enzymes demonstrated that side chain hydroxylation is catalyzed by CYP3A4, and *O*-demethylation is catalyzed by CYP2D6. No hydrolysis to indomethacin was observed in these studies, or during incubations of Compound **16** with rat liver cytosol or rat plasma. The finding that most of the metabolism of Compound **16** occurs in the amide side chain suggests that the use of more sterically hindered or electron-withdrawing substituents might improve compound stability. This was confirmed in the cases of Compounds **17** and **18**, both of which were metabolized more slowly than Compound **16** by rat liver microsomes, (half-lives of 75 minutes, and 100 minutes, respectively).

Consistent with the data obtained with rat liver microsomes, Compound **16** demonstrated poor bioavailability, a short half-life, and a low maximal plasma concentration after oral dosing in rats, although a long terminal half-life was observed after intravenous dosing. In addition to the metabolites expected from the *in vitro* studies, indomethacin was detected in the plasma of treated rats. Approximately 4% of the administered dose was converted to indomethacin.

As predicted from its slower rate of microsomal metabolism, Compound **18** proved to be the most promising of the three compounds from a metabolic perspective. It exhibits 30% oral bioavailability, a clearance half-life of 4 hours, and a very low conversion to indomethacin *in vivo* (~ 0.5% of the administered dose).

Example 14

In Vivo Anti-Inflammatory Efficacy

Despite their vast differences in pharmacokinetic parameters, both Compounds **16** and **18** are effective anti-inflammatory compounds in the rat carageenan footpad model. ED_{50} values for Compounds **16** and **18** (0.8 mg/kg and 0.25 mg/kg, respectively) indicated favorable potency for these compounds as compared to indomethacin (ED_{50} = 2 mg/kg). Although anti-inflammatory efficacy is not required for an imaging agent, the fact that these compounds have comparable or superior potency to indomethacin confirms that they reach and bind to COX-2 *in vivo*, a desirable characteristic.

Example 15

Evaluation of Monochromatic X-ray Imaging Agents

Compounds containing multiple iodine atoms can be used for monochromatic X-ray imaging. For the evaluation of these compounds, tumor-bearing and control mice are imaged with the monochromatic X-ray beam in a CT geometry both below and above the iodine K-edge. A cylindrical water bolus surrounds the mice to help attenuate the X-ray beam and to normalize exposure. The procedure is then repeated following intravenous administration of the imaging agent. The CT study is interpreted by a "blinded" radiologist to determine visibility of the tumors and any alteration in attenuation engendered by the administration of the COX-2 agent.

Example 16

Evaluation of PET Imaging Agents

For imaging, the COX-2 selective imaging agent is labeled with 0.5-1 mCi of a positron emitting agent: ^{18}F. Test animals are sedated, placed in the micro-PET system, and then imaged in dynamic 3D mode following injection. Injection volume is small (0.1-0.3 ml). Dynamic images are acquired every 5 minutes for the first hour and then serial static images are performed each 30 minutes for 3 hours. Static images are approximately 15 minutes in duration, depending upon the actual injected activity level. Time-

activity curves are generated for both normal and tumor regions and standard uptake ratio values are determined in order to quantify the degree of tumor enhancement.

Example 17

Evaluation of MRI Imaging Agents

MR imaging is performed either with a 4 cm volume coil for whole-body imaging or with a 2.5 cm (inner diameter) surface coil for implanted tumors. In all studies, the animals are imaged prior to and following the injection of the gadolinium-labeled COX-2 selective imaging agent. After injection, images are made sequentially. Images are acquired approximately every minute for 30 minutes and then every 15 minutes for a total period of 4 hours. Initially animals will be re-imaged at 24 hours. Images are analyzed using the U.S. National Institutes of Health (NIH) supplied image-analysis software package, ImageJ. Image signal-enhancement over both normal and tumor regions is quantified as both a function of time and dose level.

Example 18

Evaluation of COX-2-Selective Imaging Agents *In Vivo*:

HCA-7 Human Colon Carcinoma Xenografts

Imaging agents that target the COX-2 enzyme *in vivo* can be used to detect tumors expressing elevated levels of this enzyme. Agents that have been identified as promising using the described methods are tested *in vivo* using a number of tumor models. An exemplary model system is the HCA-7 human colon adenocarcinoma cell line. HCA-7 cells are readily cultured *in vitro*, and can be evaluated as tumor xenografts *in vivo*. They express COX-2, and it is well-documented that NSAIDs and selective COX-2 inhibitors cause a reduction in the size and number of colonies formed by these cells when grown in soft agar or matrigel. Similarly, NSAIDs and COX-2 inhibitors cause a reduction in tumor formation and growth of HCA-7 cell xenografts in nude mice (Sheng *et al.*, 1997; Williams *et al.*, 2000b; Mann *et al.*, 2001).

Tumor xenografts are established by injecting 10^6 HCA-7 cells suspended in 0.2 mL of culture medium into the dorsal subcutaneous tissue

of athymic nude mice. Measurable solid tumors are detected within 1 to 2 weeks, at which point they are suitable for imaging studies. This model is particularly useful, because tumors form quickly in a well-defined, subcutaneous location, allowing testing of all imaging modalities under nearly ideal conditions. Xenografts of HCT-116 cells, a colon cancer cell line that is not COX-2 dependent, are used as a negative control Sheng *et al.*, 1997). The HCT-116 xenografts are also used to evaluate the level of COX-2 expression in tissue surrounding the tumor, a factor that has been shown to contribute to tumor angiogenesis and growth (Williams *et al.*, 2000a).

Example 19

Evaluation of COX-2-Selective Imaging Agents *In Vivo*: Murine Lewis Lung Carcinoma

Compounds that show promise in the HCA-7 xenograft model are tested against the murine Lewis lung carcinoma cell line. This cell line provides a syngeneic tumor model that can be used in C57BL/6 mice without concern of tumor rejection. Lewis lung carcinoma cells have been shown to express COX-2 *in vitro* and *in vivo*, and the administration of NSAIDs or COX-2 inhibitors has been shown to reduce cell proliferation and viability *in vitro*, and to reduce tumorigenesis and tumor growth *in vivo* (Stolina *et al.*, 2000; Eli *et al.*, 2001). Intravenous injection of Lewis lung carcinoma cells (5 x 10^5) leads to the formation of lung tumors within 30 to 40 days. Subcutaneous injection of the cells (5 x 10^5) leads to the formation of localized solid tumors. Therefore, as in the case of the HCA-7 xenograft, this model allows the testing of well-defined subcutaneous tumors, but also provides the opportunity to evaluate compounds for imaging tumors at the more challenging intrathoracic location.

Example 20

Evaluation of COX-2-Selective Imaging Agents *In Vivo*: Murine Models of Colorectal Carcinoma

The HCA-7 and Lewis lung carcinoma models are advantageous, in that they allow the study of an imaging agent in a defined, solid tumor at a

known location. However, ultimate clinical application will require the detection of small, spontaneous tumors that arise *in situ*. Two models of colon carcinogenesis are available that will allow the evaluation of imaging agents under these circumstances, the APC^{Min-} mouse model, and the azoxymethane tumorigenesis model.

<u>APC^{Min-} Mouse Model</u>

Familial adenomatous polyposis (FAP) in humans is associated with the development of large numbers of intestinal adenomas at an early age, with progression to carcinomas over time. This condition results from mutation in the APC (adenomatous polyposis coli) gene, and a number of mouse models exist in which this gene has been altered, either by chemical exposure or by site-directed mutagenesis. The APC^{Min-} (multiple intestinal neoplasia) mouse model was developed through a chemically-induced germline mutation at codon 850 of the APC gene (Su *et al.*, 1992; Moser *et al.*, 1995). These mice develop multiple intestinal and colonic adenomas by 100 days of age. Increased expression of COX-2 has been demonstrated in the adenomas and surrounding stroma, and administration of NSAIDs and selective COX-2 inhibitors reduces both the number and size of adenoma formation (Boolbol *et al.*, 1996; Williams *et al.*, 1996; Barnes and Lee, 1998; Jacoby *et al.*, 2000). In a similar model, $APC^{\Delta 716}$, coexpression of the APC mutation with targeted deletion of the COX-2 gene resulted in a reduced number and size of adenomas when compared to expression of the APC mutation in mice normozygous for COX-2 (Oshima *et al.*, 1996).

<u>Azoxymethane-Induced Colon Carcinoma</u>

A second well-defined model of colon tumorigenesis in rodents is derived from the subcutaneous injection of azoxymethane in weanling rats or mice. In this model, azoxymethane is administered subcutaneously or intraperitoneally at weekly doses of 10 to 15 mg/kg for a period of 2 to 6 weeks. Fully developed adenocarcinomas are observed at 30 to 50 weeks after treatment. Experiments in rats have demonstrated increased expression of COX-2 in azoxymethane-induced colonic tumors when

compared to normal colonic tissue DuBois *et al.*, 1996; Jacoby *et al.*, 2000; Takahashi *et al.*, 2000; Kishimoto *et al.*, 2002a). Furthermore, NSAIDs and COX-2 inhibitors have been shown to decrease both the number and size of colonic tumors resulting from azoxymethane treatment in both rats and mice (Yoshima *et al.*, 1997; Fukutake *et al.*, 1998; Reddy *et al.*, 2000; Kishimoto *et al.*, 2002b). In order to generate tumors for use in assessing the utility of imaging agents, 6 week old male mice will be treated for 6 weeks with weekly intraperitoneal injections of 10 mg/kg azoxymethane (Fukutake *et al.* 1998).

Both the APC^{Min-} mouse model and the mouse azoxymethane-induced colon carcinoma model are used to determine the effectiveness of promising imaging agents. The azoxymethane model poses the disadvantage that over 7 months are required for tumor formation. However, because the tumors generated in this model are highly COX-2 dependent, and because the prior research in this model is extensive, this model is a valuable system in which to evaluate compounds. In both models, imaging agents are assessed at various points during disease progression in order to determine the effectiveness of each agent to detect tumors at early stages. Results are correlated with pathological evaluation of intestinal tissue.

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The references listed below as well as all references cited in the specification are incorporated herein by reference to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

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It will be understood that various details of the described subject matter can be changed without departing from the scope of the described subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

CLAIMS

What is claimed is:

1. A method for synthesizing a radiological imaging agent, the method comprising reacting a COX-2-selective ligand with a compound comprising a detectable group, wherein the COX-2-selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety.

2. The method of claim 1, wherein a carboxylic acid group of the NSAID has been derivatized to an ester or a secondary amine.

3. The method of claim 1, wherein the NSAID is selected from the group consisting of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof.

4. The method of claim 1, wherein the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, 6-methoxy-α-methyl-2-naphthylacetic acid, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolic acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zomepirac, and pharmaceutically acceptable salts thereof, and combinations thereof.

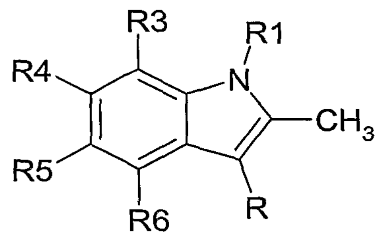
5. The method of claim 4, wherein the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), ketorolac, and pharmaceutically acceptable salts thereof, and combinations thereof.

6. The method of claim 1, wherein the secondary amide derivative is selected from the group consisting of indomethacin-*N*-methyl amide, indomethacin-*N*-ethan-2-ol amide, indomethacin-*N*-octyl amide, indomethacin-*N*-nonyl amide, indomethacin-*N*-(2-methylbenzyl) amide, indomethacin-*N*-(4-methylbenzyl) amide, indomethacin-*N*-[(R)-α,4-dimethylbenzyl] amide, indomethacin-*N*-((S)-α,4-dimethylbenzyl) amide, indomethacin-*N*-(2-phenethyl) amide, indomethacin-*N*-(4-fluorophenyl) amide, indomethacin-*N*-(4-chlorophenyl) amide, indomethacin-*N*-(4-acetamidophenyl) amide, indomethacin-*N*-(4-methylmercapto)phenyl amide, indomethacin-*N*-(3-methylmercaptophenyl) amide, indomethacin-*N*-(4-methoxyphenyl) amide, indomethacin-*N*-(3-ethoxyphenyl) amide, indomethacin-*N*-(3,4,5-trimethoxyphenyl) amide, indomethacin-*N*-(3-pyridyl) amide, indomethacin-*N*-5-[(2-chloro)pyridyl] amide, indomethacin-*N*-5-[(1-ethyl)pyrazolo] amide, indomethacin-*N*-(3-chloropropyl) amide, indomethacin-*N*-methoxycarbonylmethyl amide, indomethacin-*N*-2-(2-L-methoxycarbonylethyl) amide, indomethacin-*N*-2-(2-D-methoxycarbonylethyl) amide, indomethacin-*N*-(4-methoxycarbonylbenzyl) amide, indomethacin-*N*-(4-methoxycarbonylmethylphenyl) amide, indomethacin-*N*-(2-pyrazinyl) amide, indomethacin-*N*-2-(4-methylthiazolyl) amide, indomethacin-*N*-(4-biphenyl) amide, and combinations thereof.

7. The method of claim 1, wherein the detectable group is selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

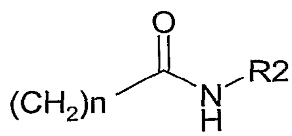
8. The method of claim 7, wherein the halogen-containing moiety comprises a chloride atom, a fluorine atom, an iodine atom, a bromine atom, or a radioactive isotope thereof.

9. The method of claim 1, wherein the radiological imaging agent comprises the following structure:

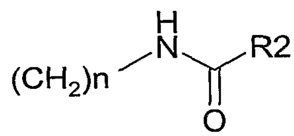


wherein

R is selected from the group consisting of

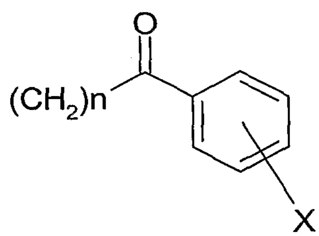


and



;

R1 is selected from the group consisting of a detectable group,



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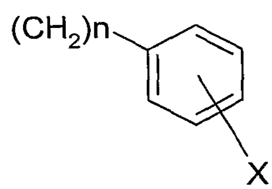


,

wherein X is a halogen or a radioactive isotope thereof at one or more positions of the aromatic ring;

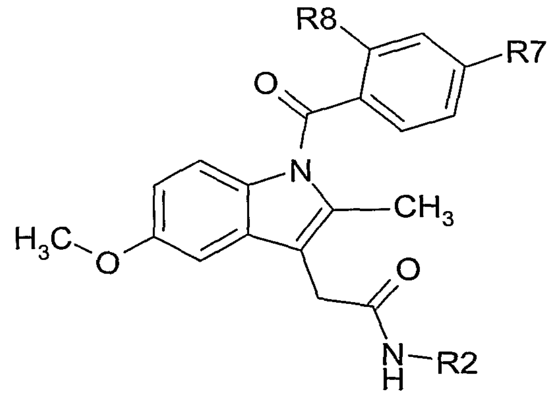
R2 comprises a detectable group or a halo substituted aryl;

R3, R4, R5, and R6 are each independently selected from the group consisting of hydrogen; halo; C_1 to C_6 alkyl or branched alkyl; C_1 to C_6 alkoxy or branched alkoxy; benzyloxy; SCH_3; $SOCH_3$; SO_2CH_3; SO_2NH_2; and $CONH_2$;

n is 0-5 inclusive;

and wherein at least one of R1 and R2 comprises a detectable group.

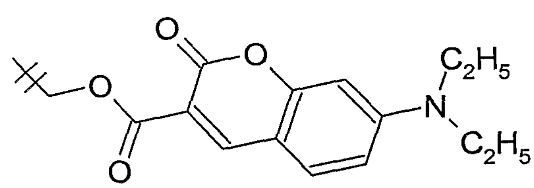
10. The method of claim 9, wherein the radiological imaging agent comprises the following structure:



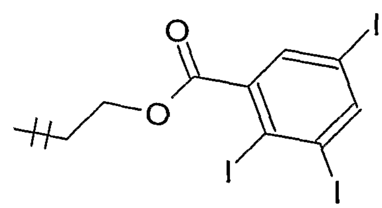
wherein R7 comprises a halogen and R8 is selected from the group consisting of hydrogen, a halogen, C_1-C_6 alkyl or branched alkyl, and C_1-C_6 aryl or branched aryl.

11. The method of claim 10, wherein at least one of R7 and R8 comprises ^{18}F.

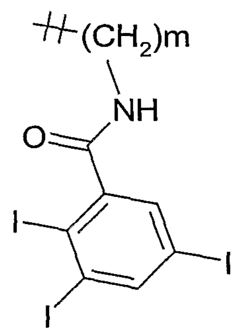
12. The method of claim 10, wherein R7 is Cl, R2 has the following structure:



13. The method of claim 10, wherein R7 is Cl and R2 has the following structure:

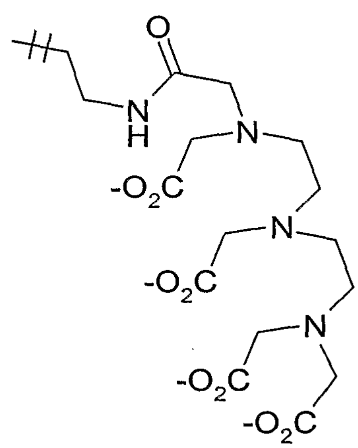


14. The method of claim 10, wherein R7 is Cl and R2 has the following structure:



wherein m = an integer between 0 and 8, inclusive.

15. The method of claim 10, wherein R7 is Cl and R2 has the following structure:

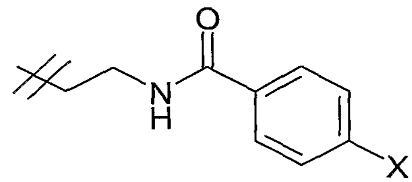


16. The method of claim 15, further comprising a coordinated metal ion.

17. The method of claim 16, wherein the coordinated metal ion is selected from the group consisting of Gd^{3+}, Eu^{3+}, Fe^{3+}, Mn^{2+}, Yt^{3+}, Dy^{3+}, and Cr^{3+}.

18. The method of claim 17, wherein the coordinated metal ion is Gd^{3+} or Eu^{3+}.

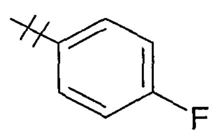
19. The method of claim 10, wherein R7 is Cl and R2 has the following structure:



and wherein X is a halogen or a radioactive isotope thereof.

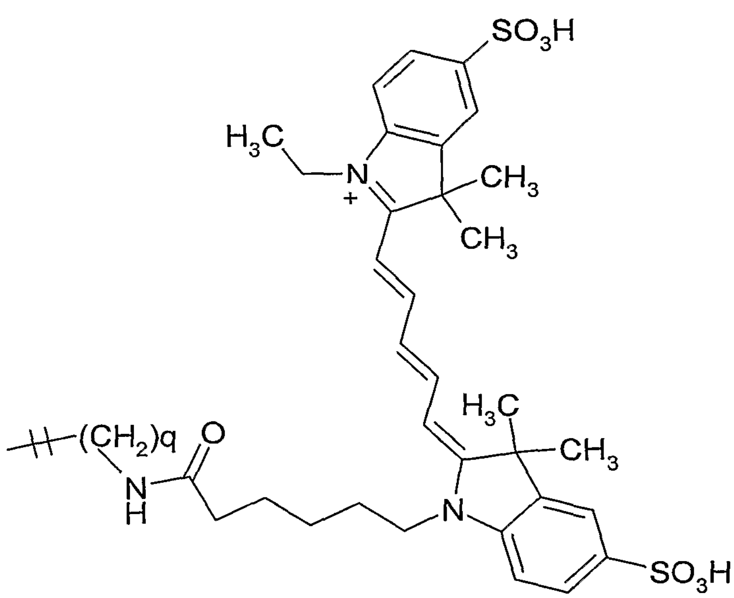
20. The method of claim 19, wherein X is ^{18}F.

21. The method of claim 10, wherein R7 is Cl and R2 has the following structure:



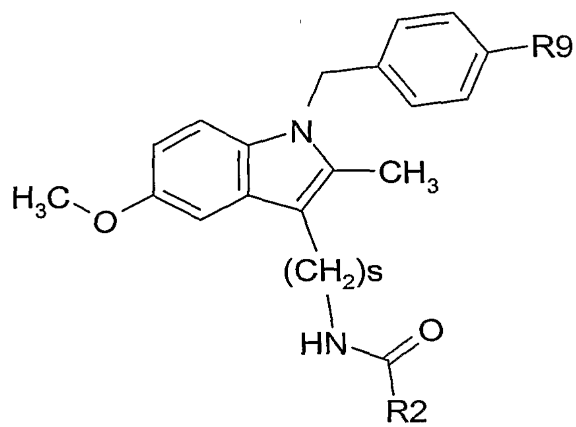
22. The method of claim 21, wherein R8 is ^{18}F.

23. The method of claim 10, wherein R7 is Cl and R2 has the following structure:



wherein q = an integer between 0 and 8, inclusive.

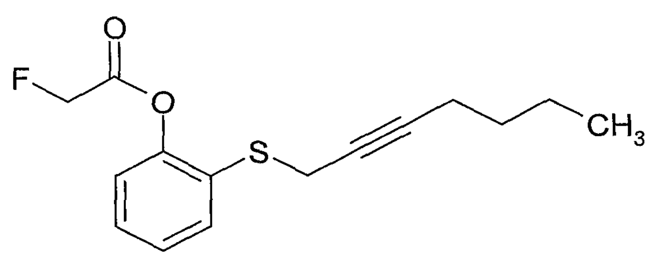
24. The method of claim 9, wherein the radiological imaging agent comprising the following structure:



wherein R9 is a halogen, R2 is *p*-halobenzene, and s = 1-4.

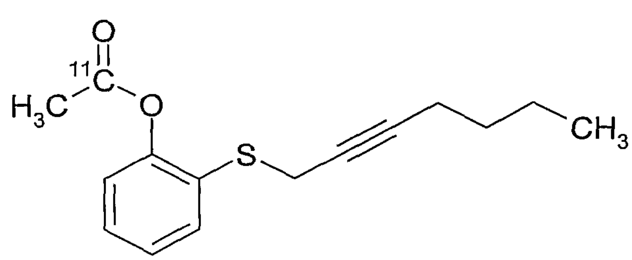
25. The method of claim 24, wherein R9 is Br, n = 2, and R2 is *p*-^{18}F-benzene.

26. The method of claim 1, wherein the radiological imaging agent comprises the following structure:

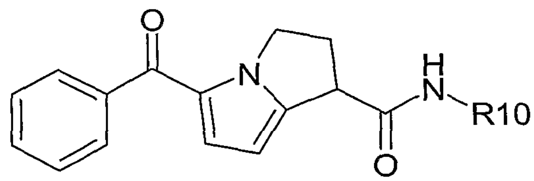


27. The method of claim 26, wherein the fluorine atom is ^{18}F.

28. The method of claim 1, wherein the radiological imaging agent comprises the following structure:

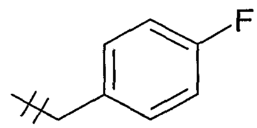


29. The method of claim 1, wherein the radiological imaging agent comprises the following structure:



wherein R comprises a detectable group.

30. The method of claim 29, wherein R10 has the following structure:



31. A method for imaging a target tissue in a subject, the method comprising:

(a) administering to the subject a radiological imaging agent under conditions sufficient for binding the radiological imaging agent to the target tissue, wherein the radiological imaging agent comprises a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety and further comprises a detectable group; and

(b) detecting the detectable group in the target tissue.

32. The method of claim 31, wherein a carboxyl group of the non-steroidal anti-inflammatory drug has been derivatized to an ester or secondary amide.

33. The method of claim 31, wherein the target tissue is selected from the group consisting of an inflammatory lesion, a tumor, a pre-neoplastic lesion, a neoplastic cell, a pre-neoplastic cell, and a cancer cell.

34. The method of claim 33, wherein the pre-neoplastic lesion is selected from the group consisting of a colon polyp and Barrett's esophagus.

35. The method of claim 33, wherein the tumor is selected from the group consisting of a primary tumor, a metastasized tumor, and a carcinoma.

36. The method of claim 31, wherein the subject is a mammal.

37. The method of claim 36, wherein the mammal is a human.

38. The method of claim 31, wherein the administering is via a route selected from the group consisting of peroral, intravenous, intraperitoneal, inhalation, and intratumoral.

39. The method of claim 30, wherein the (NSAID) is selected from the group consisting of fenamic acids, indoles, phenylalkanoic acids, phenylacetic acids, pharmaceutically acceptable salts thereof, and combinations thereof.

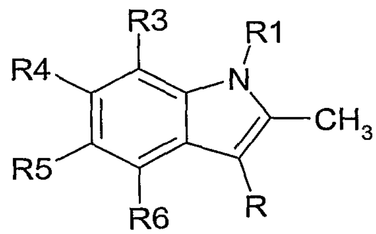
40. The method of claim 31, wherein the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, 6-methoxy-α-methyl-2-naphthylacetic acid, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), diclofenac, flufenamic acid, niflumic acid, mefenamic acid, sulindac, tolmetin, suprofen, ketorolac, flurbiprofen, ibuprofen, aceloferac, alcofenac, amfenac, benoxaprofen, bromfenac, carprofen, clidanac, diflunisal, efenamic acid, etodolic acid, fenbufen, fenclofenac, fenclorac, fenoprofen, fleclozic acid, indoprofen, isofezolac, ketoprofen, loxoprofen, meclofenamate, naproxen, orpanoxin, pirprofen, pranoprofen, tolfenamic acid, zaltoprofen, zomepirac, and pharmaceutically acceptable salts thereof, and combinations thereof.

41. The method of claim 40, wherein the NSAID is selected from the group consisting of aspirin, *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), indomethacin, meclofenamic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), ketorolac, and pharmaceutically acceptable salts thereof, and combinations thereof.

42. The method of claim 31, wherein the detectable group is selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

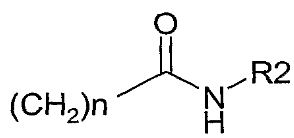
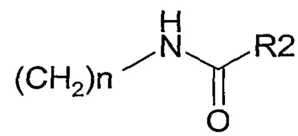
43. The method of claim 31, wherein the detecting is by positron emission tomography, near infrared luminescence, or monochromatic X-ray.

44. The method of claim 31, wherein the radiological imaging agent comprises the following structure:

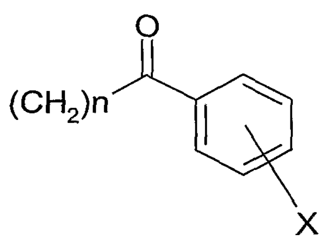
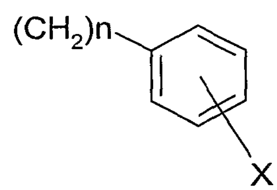


wherein

R is selected from the group consisting of

 and  ;

R1 is selected from the group consisting of a detectable group,

 , and  ,

wherein X is a halogen or a radioactive isotope thereof at one or more positions of the aromatic ring;

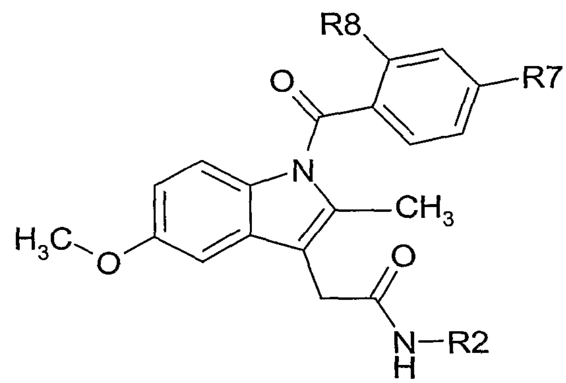
R2 comprises a detectable group or a halo substituted aryl;

R3-R6 are each independently selected from the group consisting of hydrogen; halo; C_1 to C_6 alkyl or branched alkyl; C_1 to C_6 alkoxy or branched alkoxy; benzyloxy; SCH_3; $SOCH_3$; SO_2CH_3; SO_2NH_2; and $CONH_2$;

n is 0-5 inclusive;

and wherein at least one of R1 and R2 comprises a detectable group.

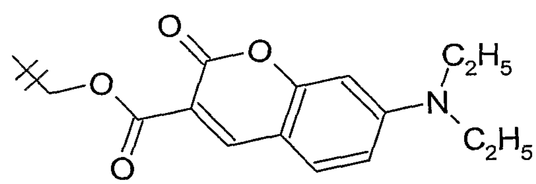
45. The method of claim 44, wherein the radiological imaging agent comprises the following structure:



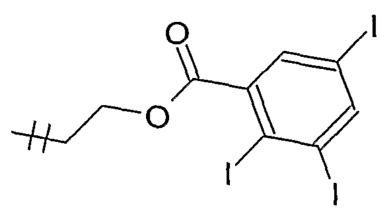
wherein R7 comprises a halogen and R3 is selected from the group consisting of hydrogen, a halogen, C_1-C_6 alkyl or branched alkyl, and C_1-C_6 aryl or branched aryl.

46. The method of claim 45, wherein at least one of R7 and R8 comprises ^{18}F.

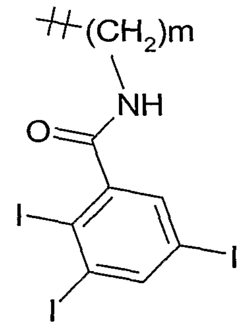
47. The method of claim 45, wherein R7 is Cl and R2 has the following structure:



48. The method of claim 45, wherein R7 is Cl and R2 has the following structure:

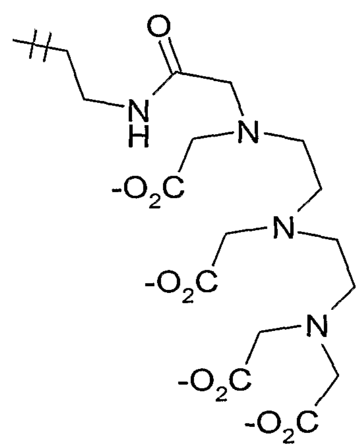


49. The method of claim 45, wherein R7 is Cl and R2 has the following structure:



wherein m = an integer between 0 and 8, inclusive.

50. The method of claim 45, wherein R7 is Cl and R2 has the following structure:

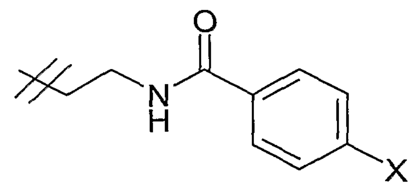


51. The method of claim 50, further comprising a coordinated metal ion.

52. The method of claim 51, wherein the coordinated metal ion is selected from the group consisting of Gd^{3+}, Eu^{3+}, Fe^{3+}, Mn^{2+}, Yt^{3+}, Dy^{3+}, and Cr^{3+}.

53. The method of claim 52, wherein the coordinated metal ion is Gd^{3+} or Eu^{3+}.

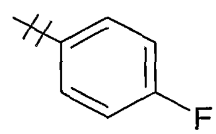
54. The method of claim 45, wherein R7 is Cl and R2 has the following structure:



and wherein X is a halogen or a radioactive isotope thereof.

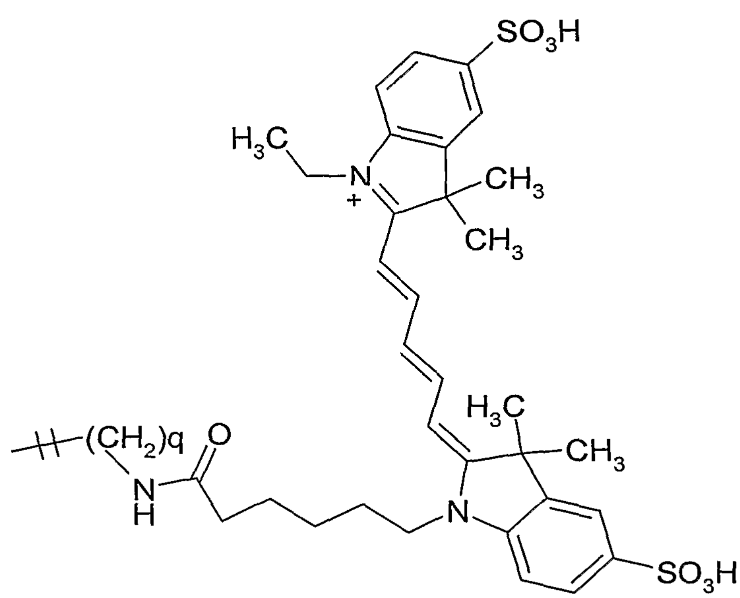
55. The method of claim 54, wherein X is ^{18}F.

56. The method of claim 45, wherein R7 is Cl and R2 has the following structure:



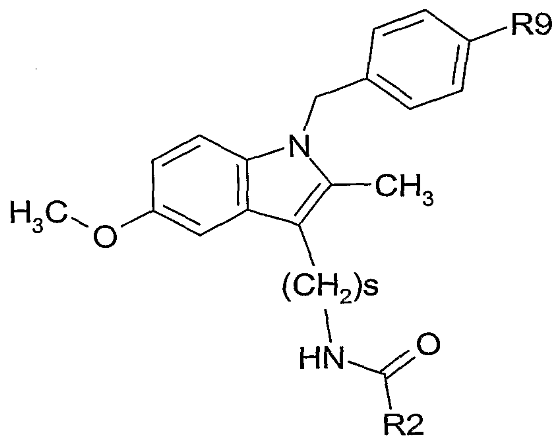
57. The method of claim 56, wherein R8 is ^{18}F.

58. The method of claim 45, wherein R7 is Cl and R2 has the following structure:



wherein q = an integer between 0 and 8, inclusive.

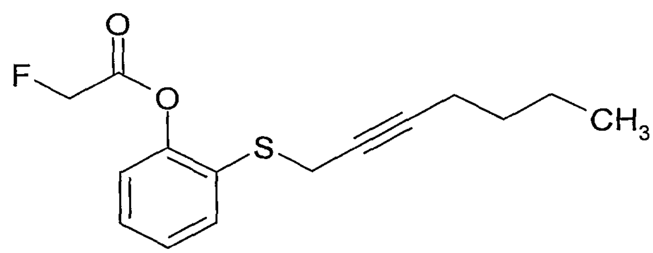
59. The method of claim 44, wherein the radiological imaging agent comprising the following structure:



wherein R1 is a halogen, R2 is *p*-halobenzene, and s = 1-4.

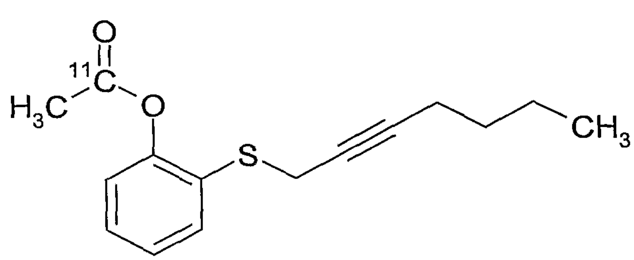
60. The method of claim 59, wherein R9 is Br, n = 2, and R2 is *p*-^{18}F-benzene.

61. The method of claim 31, wherein the radiological imaging agent comprises the following structure:

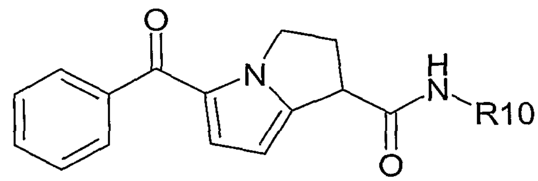


62. The method of claim 61, wherein the fluorine atom is ^{18}F.

63. The method of claim 31, wherein the radiological imaging agent comprises the following structure:

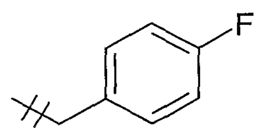


64. The method of claim 31, wherein the radiological imaging agent comprises the following structure:



wherein R comprises a detectable group.

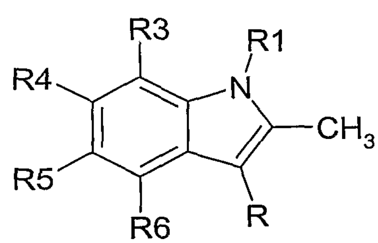
65. The method of claim 64, wherein R10 has the following structure:



66. A radiological imaging agent comprising a detectable group and a COX-2-selective ligand, wherein the ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety.

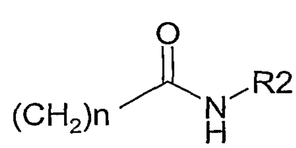
67. The radiological imaging agent of claim 66, wherein a carboxyl group of the non-steroidal anti-inflammatory drug has been derivatized to an ester or secondary amide.

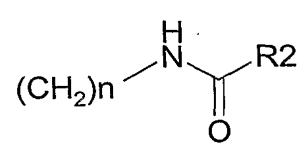
68. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:



wherein

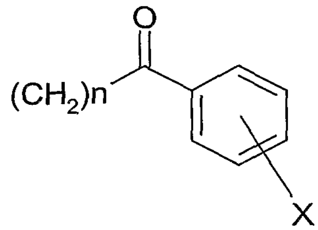
R is selected from the group consisting of



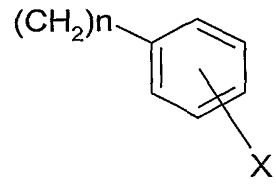
and


;

R1 is selected from the group consisting of a detectable group,



, and



,

wherein X is a halogen or a radioactive isotope thereof at one or more positions of the aromatic ring;

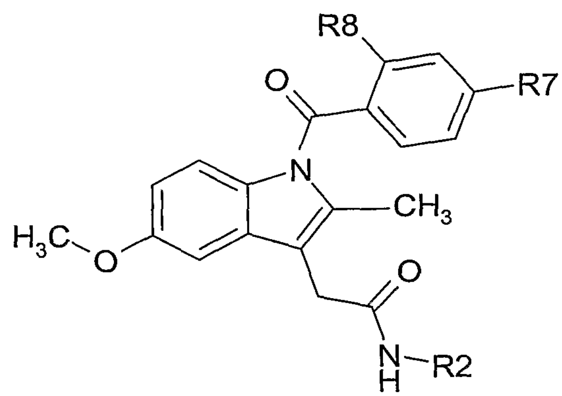
R2 comprises a detectable group or a halo substituted aryl;

R3-R6 are each independently selected from the group consisting of hydrogen; halo; C_1 to C_6 alkyl or branched alkyl; C_1 to C_6 alkoxy or branched alkoxy; benzyloxy; SCH_3; $SOCH_3$; SO_2CH_3; SO_2NH_2; and $CONH_2$;

n is 0-5 inclusive;

and wherein at least one of R1 and R2 comprises a detectable group.

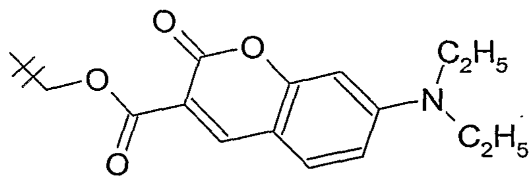
69. The radiological imaging agent of claim 68, wherein the radiological imaging agent comprises the following structure:



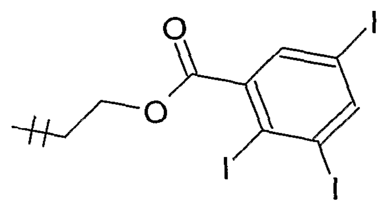
wherein R7 comprises a halogen and R3 is selected from the group consisting of hydrogen, a halogen, C_1-C_6 alkyl or branched alkyl, and C_1-C_6 aryl or branched aryl.

70. The method of claim 69, wherein at least one of R7 and R8 comprises ^{18}F.

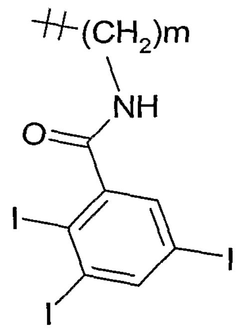
71. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:



72. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:

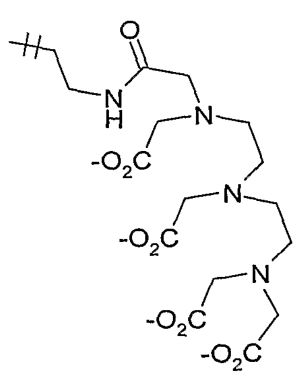


73. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:



wherein m = an integer between 0 and 8, inclusive.

74. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:

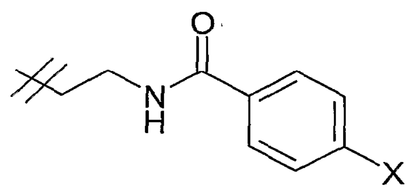


75. The radiological imaging agent of claim 74, further comprising a coordinated metal ion.

76. The radiological imaging agent of claim 75, wherein the coordinated metal ion is selected from the group consisting of Gd^{3+}, Eu^{3+}, Fe^{3+}, Mn^{2+}, Yt^{3+}, Dy^{3+}, and Cr^{3+}.

77. The radiological imaging agent of claim 76, wherein the coordinated metal ion is Gd^{3+} or Eu^{3+}.

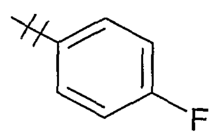
78. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:



and wherein X is a halogen or a radioactive isotope thereof.

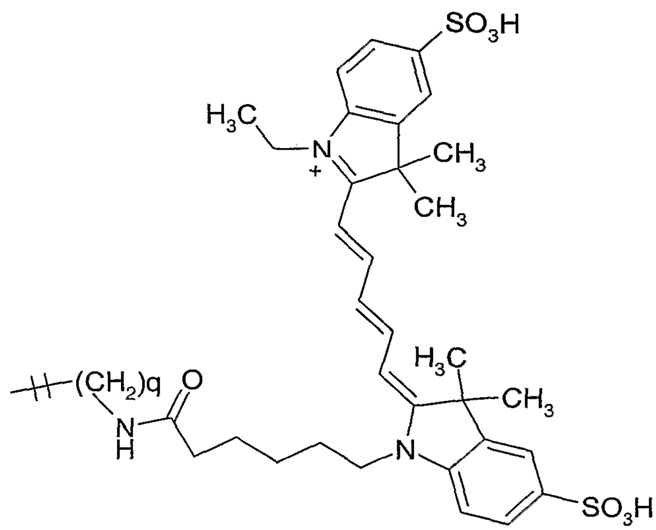
79. The radiological imaging agent of claim 78, wherein X is ^{18}F.

80. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:



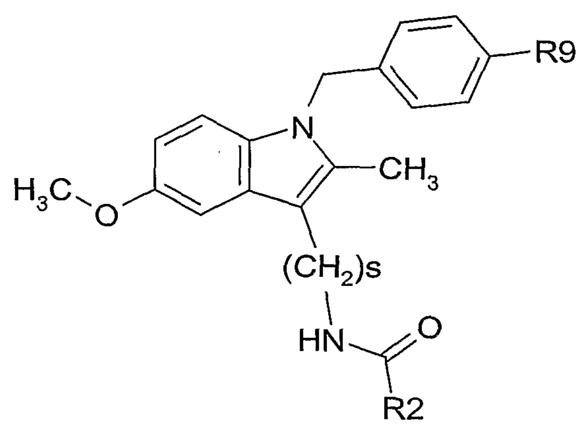
81. The radiological imaging agent of claim 80, wherein R8 is ^{18}F.

82. The radiological imaging agent of claim 69, wherein R7 is Cl and R2 has the following structure:



wherein q = an integer between 0 and 8, inclusive.

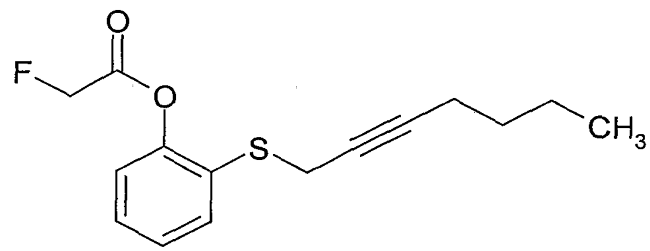
83. The radiological imaging agent of claim 68, wherein the radiological imaging agent comprising the following structure:



wherein R1 is a halogen, R2 is *p*-halobenzene, and s = 1-4.

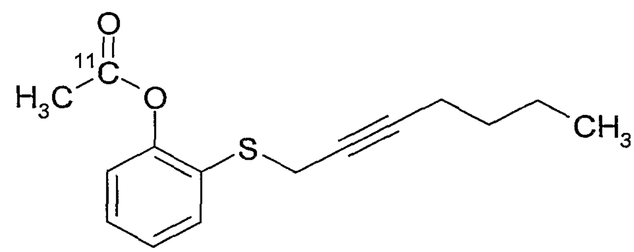
84. The radiological imaging agent of claim 83, wherein R9 is Br, n = 2, and R2 is *p*-^{18}F-benzene.

85. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:

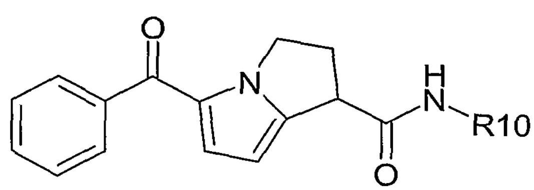


86. The radiological imaging agent of claim 85, wherein the fluorine atom is ^{18}F.

87. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:

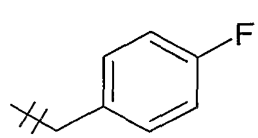


88. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:

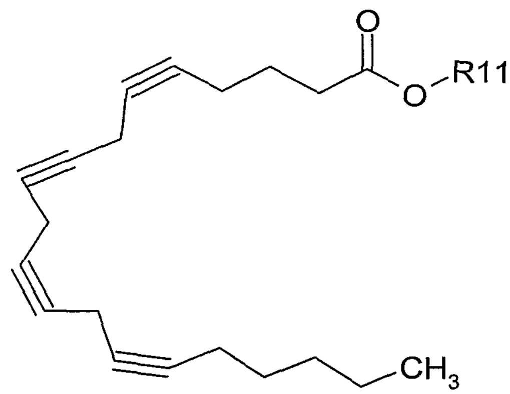


wherein R comprises a detectable group.

89. The radiological imaging agent of claim 88, wherein R10 has the following structure:

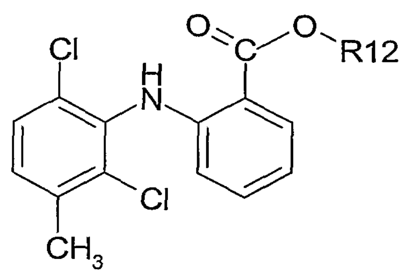


90. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:



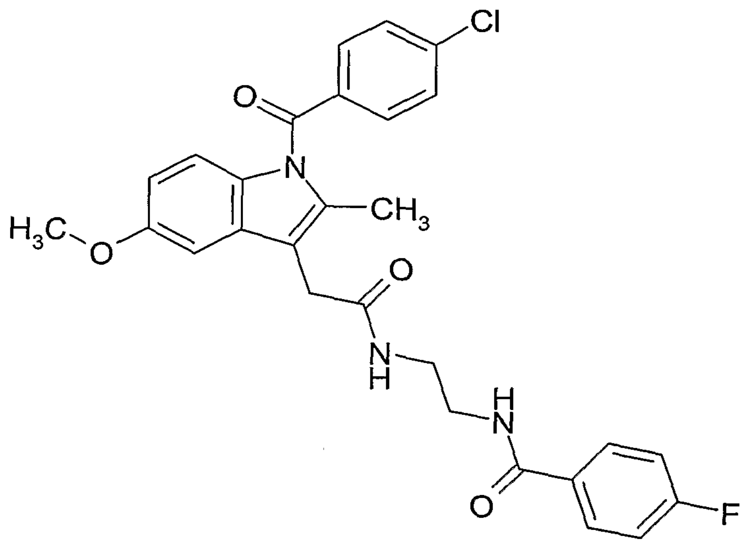
wherein R11 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

91. The radiological imaging agent of claim 66, wherein the radiological imaging agent comprises the following structure:

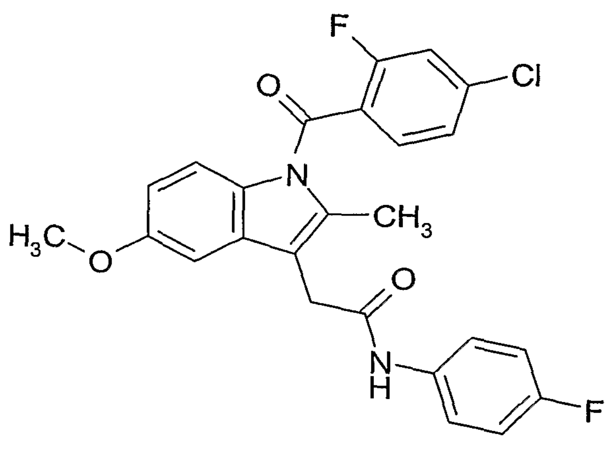


wherein R12 comprises a detectable group selected from the group consisting of a halogen-containing moiety, a fluorescent moiety, a metal ion-chelating moiety, a dye, a radioisotope-containing moiety, and combinations thereof.

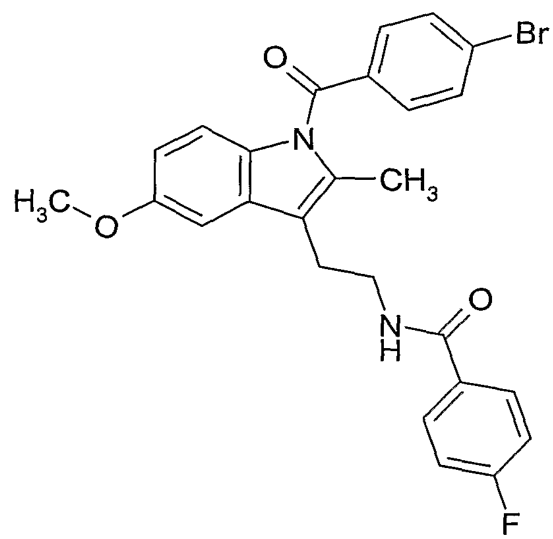
92. A radiological imaging agent comprising a detectable group and an indomethacin derivative selected from the group consisting of:



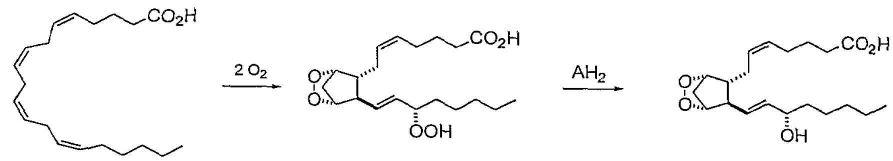
,



, and

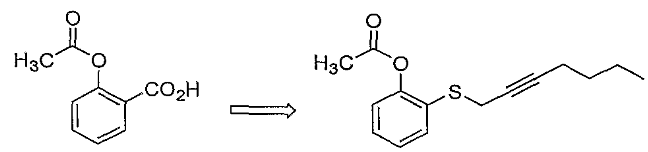


93. The radiological imaging agent of claim 92, wherein one or more fluorine atoms present is ^{18}F.



Arachidonic Acid PGG$_2$ PGH$_2$

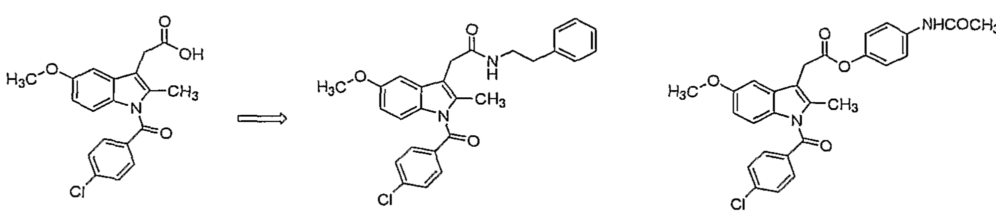
FIGURE 1



Aspirin
COX-2/COX-1 = 0.2

APHS
COX-2/COX-1 = 20

FIGURE 2

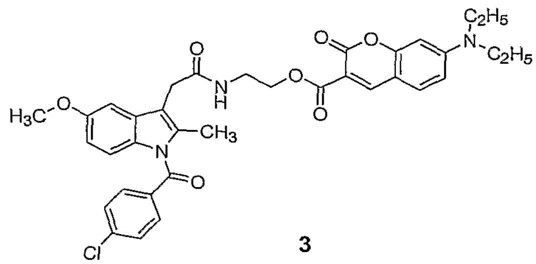


Indomethacin
COX-2/COX-1 = 0.07

1
COX-2/COX-1 = 1000

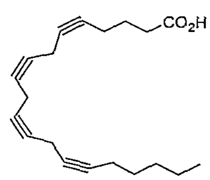
2
COX-2/COX-1 = 1300

FIGURE 3

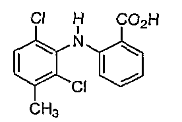


3

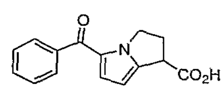
FIGURE 4



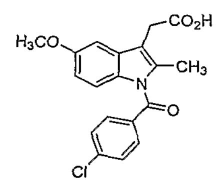
ETYA



Meclofenamic Acid



Ketorolac



Indomethacin

FIGURE 5

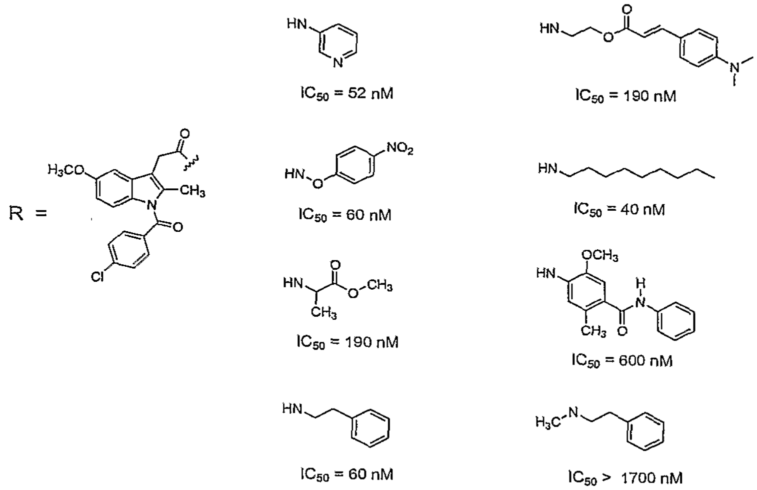


FIGURE 6

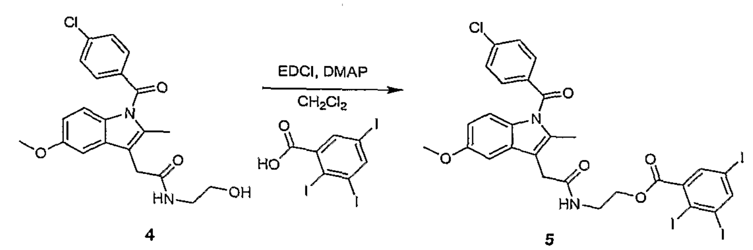


FIGURE 7

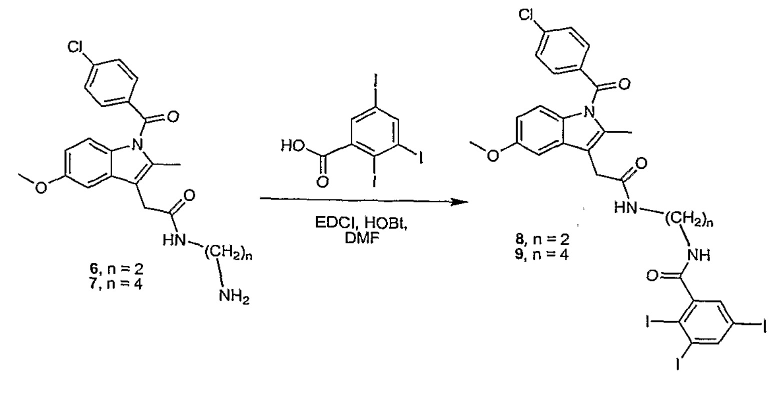


FIGURE 8

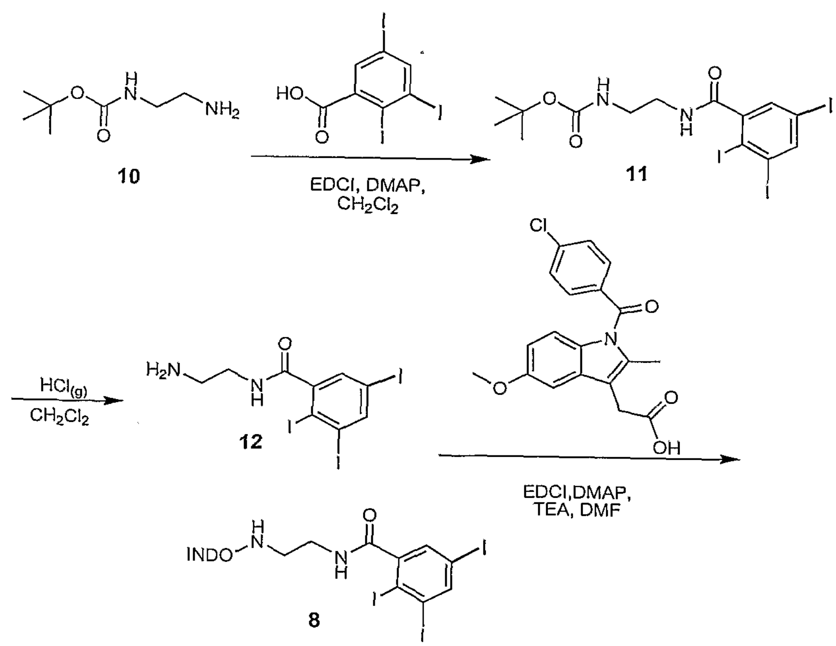


FIGURE 9

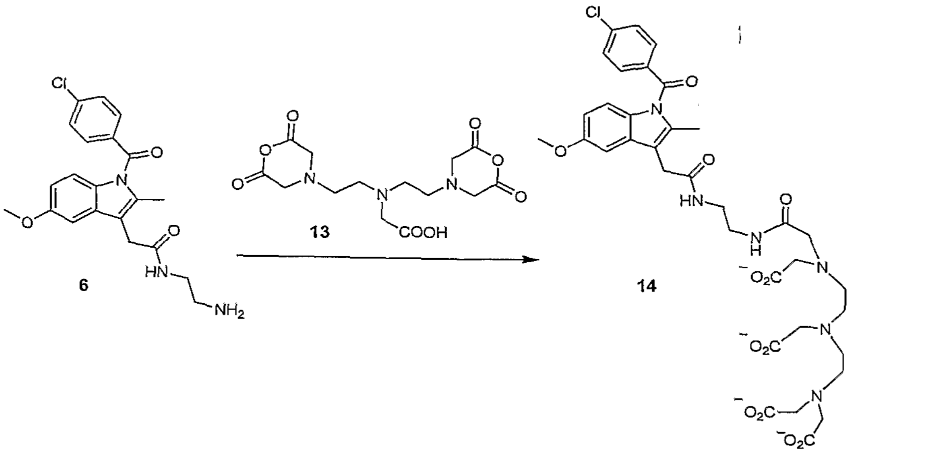


FIGURE 10

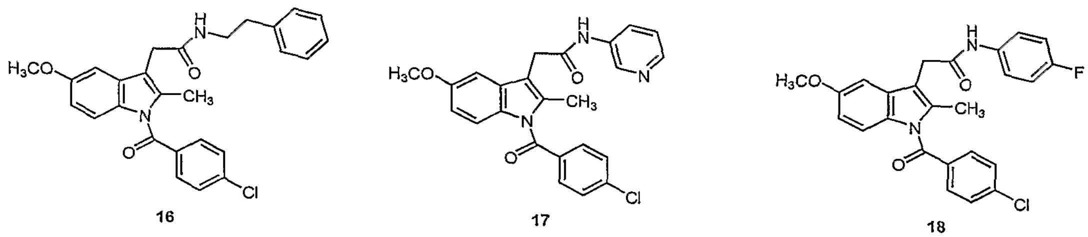


FIGURE 11

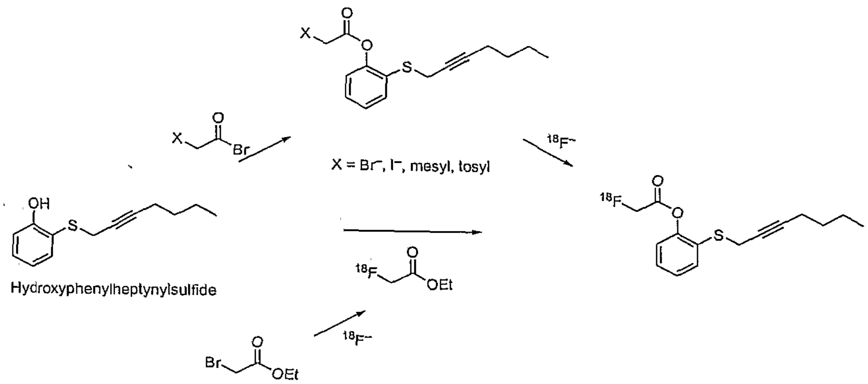


FIGURE 12

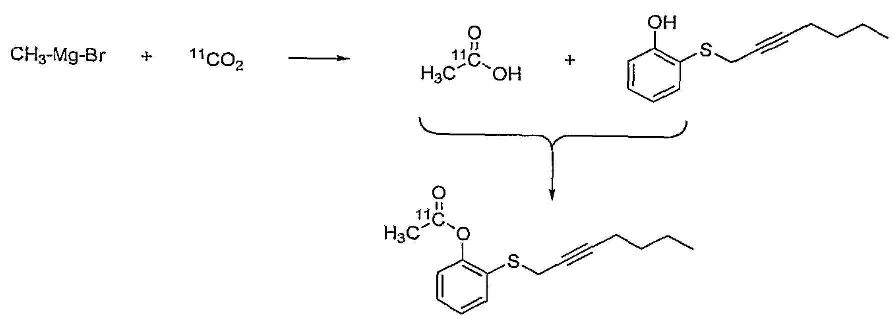
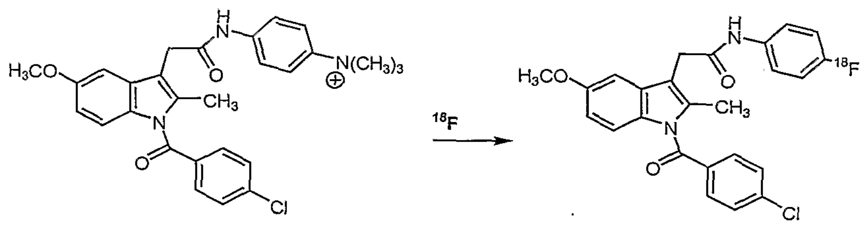
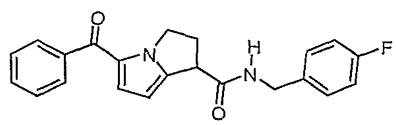


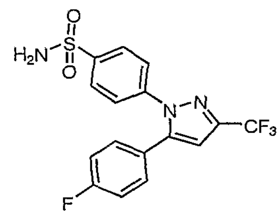
FIGURE 13



21 18

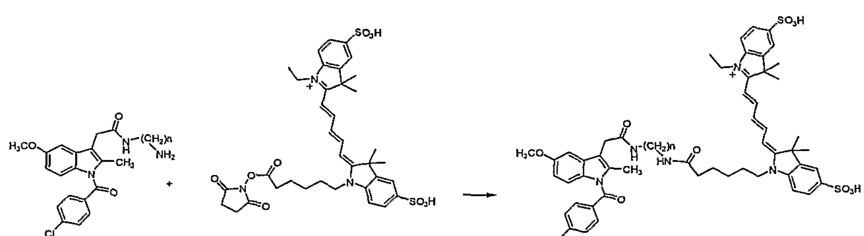


19



20

FIGURE 14



6, n = 2
7, n = 4

24, n = 2
25, n = 4

FIGURE 15

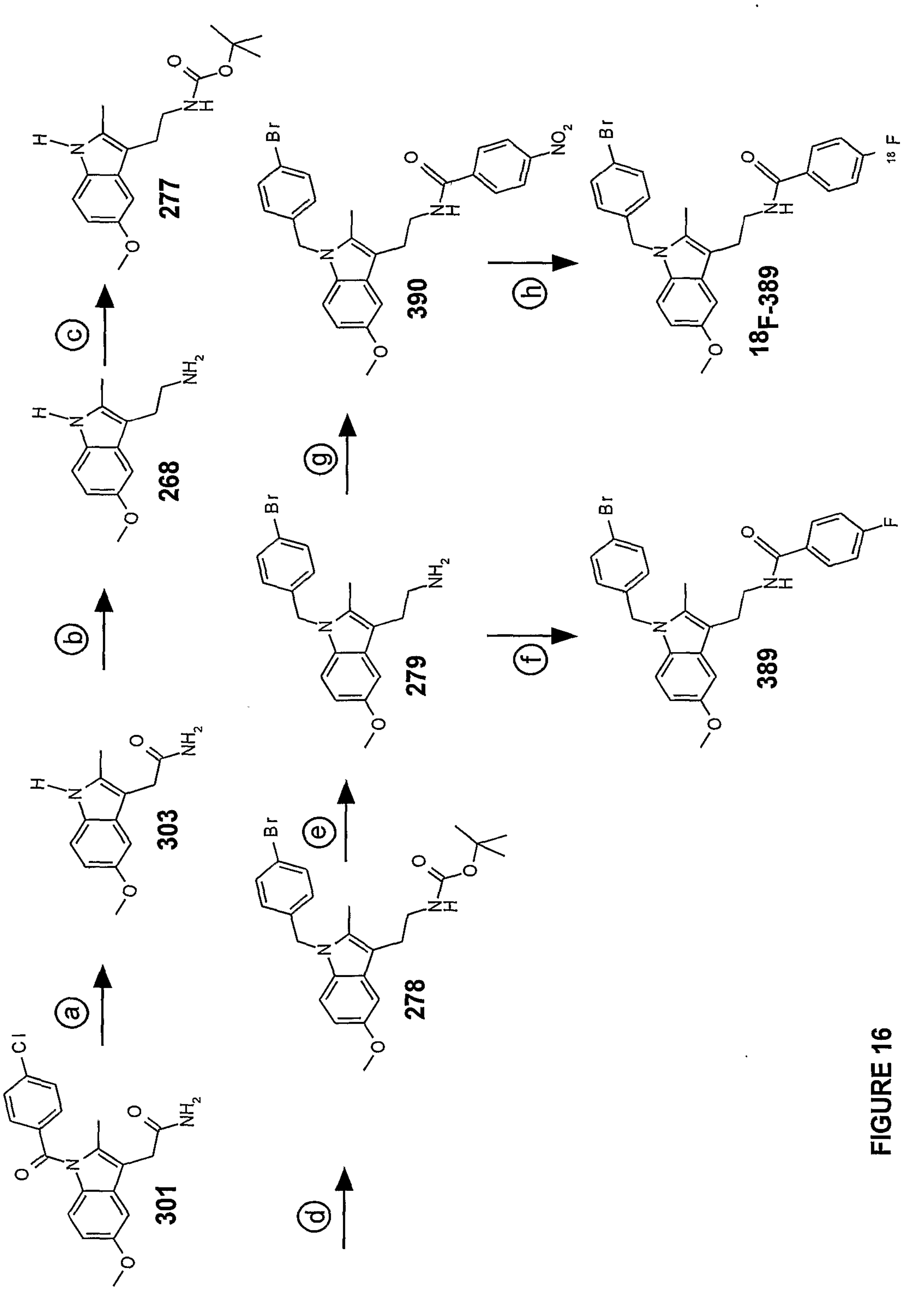

301
a
303
b
268
c
277
d
278
e
279
g
390
f
389
h
18F-389
Cl
Br
NH2
NO2
F
18 F
N
H
O

FIGURE 16

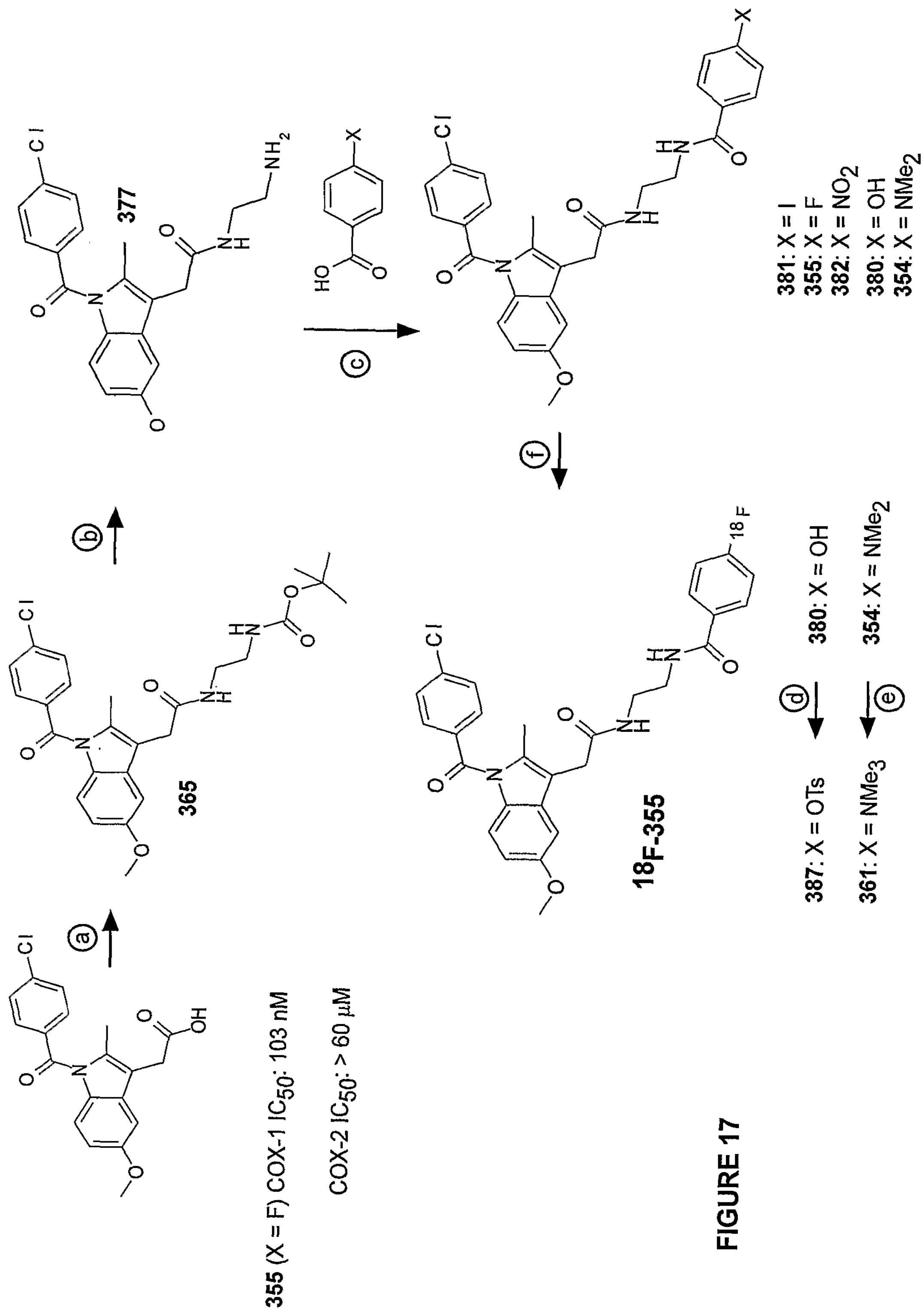

355 (X = F) COX-1 IC_{50}: 103 nM
COX-2 IC_{50}: > 60 μM
a
365
b
377
c
381: X = I
355: X = F
382: X = NO_2
380: X = OH
354: X = NMe_2
f
^{18}F-355
d
e
387: X = OTs
361: X = NMe_3
380: X = OH
354: X = NMe_2

FIGURE 17

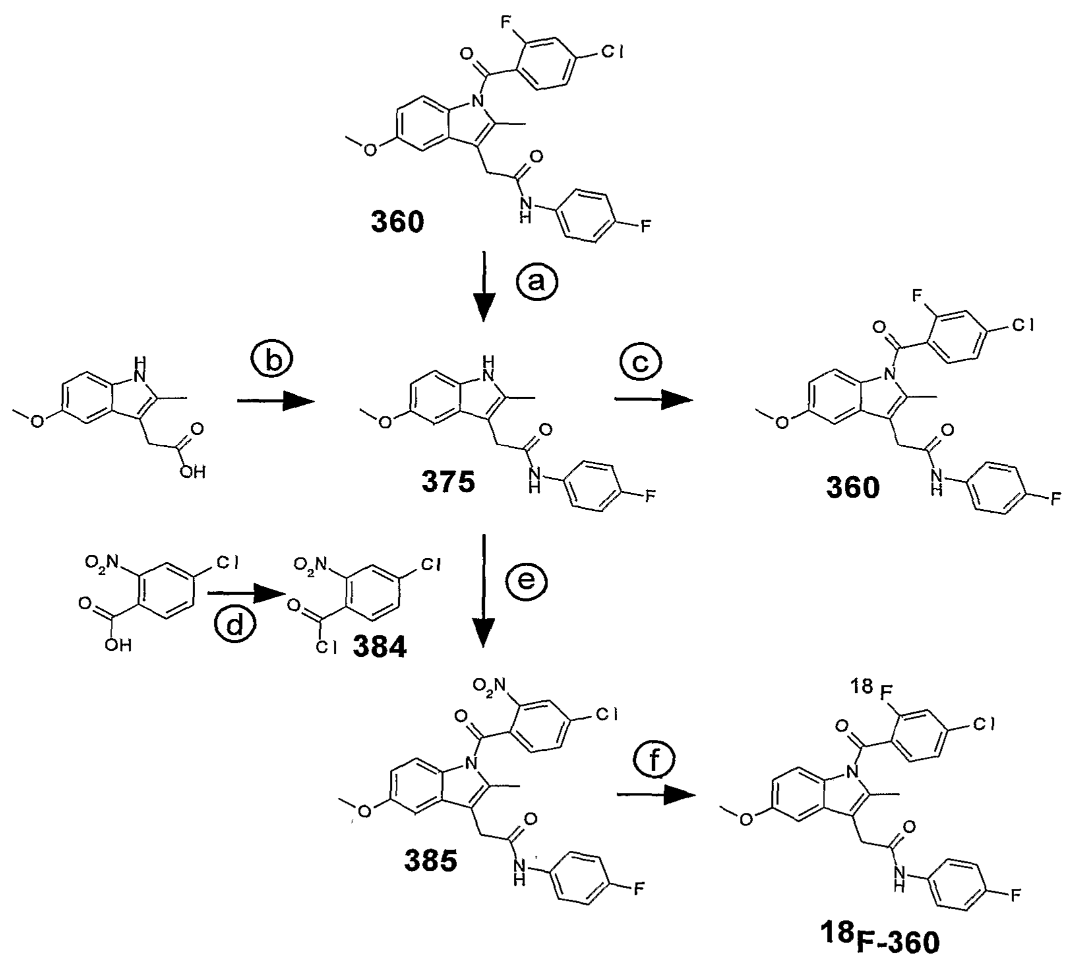
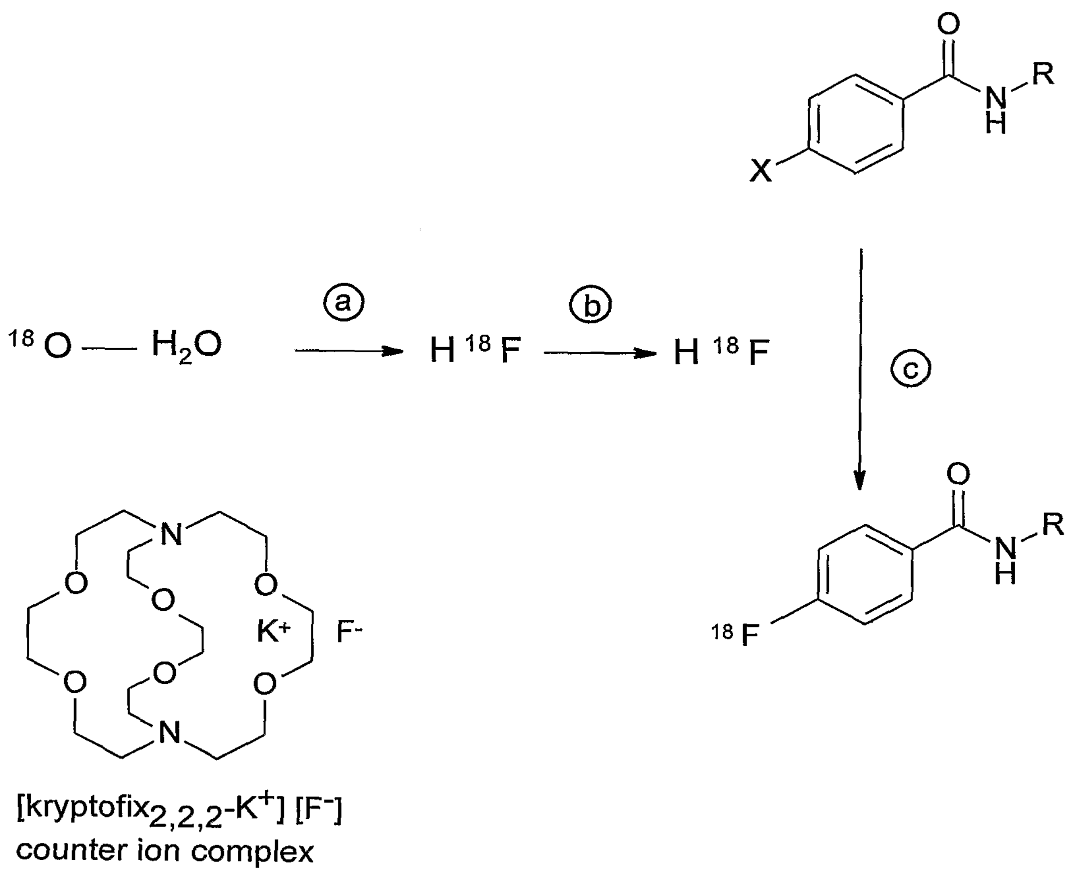


FIGURE 18



X
O
N
H
R
a
b
c
^{18}O — H_2O
$H^{18}F$
$H^{18}F$
^{18}F
O
N
H
R
N
O
O
O
K+
F-
O
O
O
N
[kryptofix$_{2,2,2}$-K$^+$] [F$^-$]
counter ion complex

FIGURE 19

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 January 2005 (06.01.2005)

PCT

(10) International Publication Number
WO 2005/002293 A3

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(21) International Application Number: PCT/US2004/020455

(22) International Filing Date: 25 June 2004 (25.06.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/482,422 25 June 2003 (25.06.2003) US

(71) Applicant *(for all designated States except US)*: **VANDERBILT UNIVERSITY** [US/US]; 1207 17th Avenue South, Suite 105, Nashville, TN 37212 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MARNETT, Lawrence, J.** [US/US]; 1884 Laurel Ridge Drive, Nashville, TN 37215 (US). **TIMOFEEVSKI, Sergei** [US/US]; 7790 Calle Mejor, Carlsbad, CA 92009 (US). **PRUDHOMME, Daniel** [US/US]; 4004 Westlawn Drive, Nashville, TN 37209 (US).

(74) Agent: **TAYLOR, Arles, A., Jr.**; Jenkins, Wilson & Taylor, P.A., Suite 1400 University Tower, 3100 Tower Boulevard, Durham, NC 27707 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 28 April 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/002293 A3

(54) **Title:** COX-2-TARGETED IMAGING AGENTS

(57) **Abstract:** The presently disclosed subject matter provides a method for synthesizing a radiological imaging agent by reacting a COX-2-selective ligand with a compound comprising a detectable group, wherein the COX-2- selective ligand is a derivative of a non-steroidal anti-inflammatory drug (NSAID) comprising an ester moiety or a secondary amide moiety. Also provided are compositions that are synthesized using the method, as well as methods of using the compositions of the presently disclosed subject matter.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/20455

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 49/00
US CL : 424/9.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/9.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
registry and caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	KUMAR, J. S. D. et al. Synthesis of [11C]-TMI: A potential PET tracer for imaging COX-2 expression. Abstracts of Papers, 228th ACS National Meeting, Philadelphia, PA, United States, August 22-26, 2004, see entire document.	1, 3-10, 24, 25, 31-38, 42-45, 59, 60, 66-70, 83, and 91 (all claims in part)

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
07 December 2004 (07.12.2004)

Date of mailing of the international search report
22 FEB 2005

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
D. L. Jones
Telephone No. (703) 308-1235

Continuation of Box II Reason 2:
The claims are directed to a radiological imaging agent comprising a COX2-selective ligand and a compound comprising a detectable group and methods of making and uses thereof. The large number of possible permutations and combinations make it virtually impossible to determine the full scope for which protection is sought. As presented, the claimed subject matter cannot be regarded as being a concise description for which protection is sought and as such, the claims do not comply with the requirements of PCT Article 6. Thus, it is impossible to perform a meaningful and timely search on the invention. A search will be provided on the first discernible invention which is the imaging agent on page 47, 18F-labeled Compound 389.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/20455

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1 (in part), 2, 3-10 (in part), 11-23, 24 (in part), 25 (in part), 26-30, 31-38 (in part), 39-41, 42-45 (in part), 46-58, 59 (in part), 60 (in part), 61-65, 66-70 (in part), 71-82, 83 (in part), 84-90, 91 (in part), 92, and 93
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.